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Amiloride, fluoxetine or riluzole to reduce brain volume loss in secondary progressive multiple sclerosis: the MS-SMART four-arm RCT

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Amiloride, fluoxetine or riluzole to reduce brain volume loss in secondary progressive multiple sclerosis: the MS-SMART four-arm RCT

Floriana De Angelis, Peter Connick, Richard A Parker, Domenico Plantone, Anisha Doshi, Nevin John, Jonathan Stutters, David MacManus, Ferran Prados, Ian Marshall, Bhavana Solanky, Rebecca S Samson, Frederik Barkhof, Sebastien Ourselin, Marie Braisher, Moira Ross, Gina Cranswick, Sue H Pavitt, Sharmilee Gnanapavan, Gavin Giovannoni, Claudia AM Gandini Wheeler-Kingshott, Clive Hawkins, Basil Sharrack, Roger Bastow, Christopher J Weir, Nigel Stallard, Siddharthan Chandran and Jeremy Chataway on behalf of The UK MS Society Clinical Trials Network for the MS-SMART Investigators



Amiloride, fluoxetine or riluzole to reduce brain volume loss in secondary progressive multiple sclerosis: the MS-SMART four-arm RCT

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Abstract

Amiloride, fluoxetine or riluzole to reduce brain volume loss in secondary progressive multiple sclerosis: the MS-SMART four-arm RCT

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Background: Neuroprotective drugs are needed to slow or prevent neurodegeneration and disability accrual in secondary progressive multiple sclerosis. Amiloride, fluoxetine and riluzole are repurposed drugs with potential neuroprotective effects.

Objectives: To assess whether or not amiloride, fluoxetine and riluzole can reduce the rate of brain volume loss in people with secondary progressive multiple sclerosis over 96 weeks. The secondary objectives that were assessed were feasibility of a multiarm trial design approach, evaluation of anti-inflammatory effects, clinician- and patient-reported efficacy and three mechanistic substudies.

Design: A multicentre, multiarm, randomised, double-blind, placebo-controlled, parallel-group Phase IIb trial with follow-up at 4, 8, 12, 24, 36, 48, 72 and 96 weeks. Patients, investigators (including magnetic resonance imaging analysts), and treating and independent assessing neurologists were blinded to the treatment allocation. The target sample size was 440 patients.

Setting: Thirteen UK clinical neuroscience centres.

Participants: Participants were aged 25–65 years, had secondary progressive multiple sclerosis with evidence of disease progression independent of relapses in the previous 2 years, and had an Expanded Disability Status Scale score of 4.0–6.5. Patients were ineligible if they could not have a magnetic resonance imaging scan; had a relapse or steroids in the previous 3 months; or had epilepsy, depression, bipolar disorder, glaucoma, bleeding disorders or significant organ comorbidities. Exclusion criteria were concurrent disease-modified treatments, immunosuppressants or selective serotonin reuptake inhibitors.

Interventions: Participants received amiloride (5 mg), fluoxetine (20 mg), riluzole (50 mg) or placebo (randomised 1 : 1 : 1 : 1) twice daily.

Main outcome measures: The primary end point was magnetic resonance imaging-derived percentage brain volume change at 96 weeks. Secondary end points were new/enlarging T2 lesions, pseudoatrophy, and clinician- and patient-reported measures (including the Expanded Disability Status Scale, Multiple Sclerosis Functional Composite, Symbol Digit Modalities Test, low-contrast letter visual acuity, Multiple Sclerosis Impact Scale 29 items, version 2, Multiple Sclerosis Walking Scale, version 2, and questionnaires addressing pain and fatigue). The exploratory end points included measures of persistent new T1 hypointensities and grey matter volume changes. The substudies were advanced magnetic resonance imaging, optical coherence tomography and cerebrospinal fluid analyses.

Results: Between December 2014 and June 2016, 445 patients were randomised (analysed) to amiloride [$n = 111$ (99)], fluoxetine [$n = 111$ (96)], riluzole [$n = 111$ (99)] or placebo [$n = 112$ (99)]. A total of 206 randomised patients consented to the advanced magnetic resonance imaging substudy, 260 consented to the optical coherence tomography substudy and 70 consented to the cerebrospinal fluid substudy. No significant difference was seen between the active drugs and placebo in percentage brain volume change at week 96 as follows (where negative values mean more atrophy than placebo): amiloride minus placebo 0.0% (Dunnett-adjusted 95% confidence interval –0.4% to 0.5%), fluoxetine minus placebo –0.1% (Dunnett-adjusted 95% confidence interval –0.5% to 0.3%); riluzole minus placebo –0.1% (Dunnett-adjusted 95% confidence interval –0.6% to 0.3%). There was good adherence to study drugs. The proportion of patients experiencing adverse events was similar in the treatment and placebo groups. There were no emergent safety issues.

Limitations: There was a lower than expected uptake in the cerebrospinal fluid substudy.

Conclusions: A multiarm Phase II paradigm is efficient in determining which neuroprotective agents to take through to Phase III trials. Amiloride, fluoxetine and riluzole were not effective in reducing the brain atrophy rate in people with secondary progressive multiple sclerosis. Mechanistic pathobiological insight was gained.

Future work: To use the information gained from the Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial (MS-SMART) to inform future trial design as new candidate agents are identified.

Trial registration: Current Controlled Trials ISRCTN28440672, NCT01910259 and EudraCT 2012-005394-31.

Funding: This project was funded by the Efficacy and Mechanism Evaluation (EME) programme, a Medical Research Council and National Institute for Health Research (NIHR) partnership. This will be published in full in *Efficacy and Mechanism Evaluation*; Vol. 7, No. 3. See the NIHR Journals Library website for further project information. This trial also received funding from the UK MS Society and the US National Multiple Sclerosis Society.

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List of supplementary material

Report Supplementary Material 1 Supplementary data

Supplementary material can be found on the NIHR Journals Library report page (<https://doi.org/10.3310/eme07030>).

Supplementary material has been provided by the authors to support the report and any files provided at submission will have been seen by peer reviewers, but not extensively reviewed. Any supplementary material provided at a later stage in the process may not have been peer reviewed.

List of abbreviations

3D	three-dimensional	GFAP	glial fibrillary acidic protein
9HPT	9-Hole Peg Test	Glx	combined glutamate and glutamine
AD	axial diffusivity	GM	grey matter
AE	adverse event	GP	general practitioner
AMD	adjusted mean difference	HCVA	high-contrast visual acuity
ART	automatic real time	IPL	inner plexiform layer
ASIC	acid-sensing ion channel	ITT	intention to treat
BDI-II	Beck Depression Inventory, version 2	MAR	missing at random
b.i.d.	bis in die (twice a day)	MD	mean diffusivity
BPI	Brief Pain Inventory	MedDRA	Medical Dictionary for Regulatory Activities
BV	brain volume	MMP9	matrix metalloproteinase 9
CD14	cluster of differentiation 14	MNAR	missing not at random
CGM	cortical grey matter	MRC	Medical Research Council
CGMV	cortical grey matter volume	MRI	magnetic resonance imaging
CI	confidence interval	MRS	magnetic resonance spectroscopy
CNS	central nervous system	MS	multiple sclerosis
CONSORT	Consolidated Standards of Reporting Trials	MSFC	Multiple Sclerosis Functional Composite
CSF	cerebrospinal fluid	MSIS29v2	Multiple Sclerosis Impact Scale 29 items, version 2
DGM	deep grey matter	MS-SMART	Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial
DGMV	deep grey matter volume	MSWSv2	Multiple Sclerosis Walking Scale, version 2
DMC	Data Monitoring Committee	MToff	magnetisation transfer off
DMT	disease-modifying therapy	MTon	magnetisation transfer on
DTI	diffusion tensor imaging	MTR	magnetisation transfer ratio
ECTU	Edinburgh Clinical Trials Unit	MUCCA	mean upper cord cross-sectional area
EDSS	Expanded Disability Status Scale	NAA	N-acetylaspartate
EQ-5D-5L	EuroQol-5 Dimensions, five-level version	NAWM	normal-appearing white matter
ETDRS	Early Treatment Diabetic Retinopathy Study		
FA	fractional anisotropy		
GCL	ganglion cell layer		

LIST OF ABBREVIATIONS

NCAM	neural cell adhesion molecule	PWSRD	peak width of skeletonised radial diffusivity
NfH	neurofilament heavy	RD	radial diffusivity
NFI	Neurological Fatigue Index	REC	Research Ethics Committee
NfL	neurofilament light	RNFL	retinal nerve fibre layer
NIHR	National Institute for Health Research	RRMS	relapsing–remitting multiple sclerosis
NMDA	N-methyl-D-aspartate	SAE	serious adverse event
NPRS	Numeric Pain Rating Scale	SDMT	Symbol Digit Modalities Test
NPS	Neuropathic Pain Scale	Semi-LASER	semi-localisation by adiabatic selective refocusing
OCT	optical coherence tomography	SIENA	Structural Image Evaluation using Normalization of Atrophy
PASAT	Paced Auditory Serial Addition Test	SLCVA	Sloan Low Contrast Visual Acuity
PBVC	percentage brain volume change	SPMS	secondary progressive multiple sclerosis
PD	proton density	SSRI	selective serotonin reuptake inhibitor
PIL	patient information leaflet	SUSAR	suspected unexpected serious adverse reaction
PPI	patient and public involvement	T25FW	Timed-25-Foot Walk
PPMS	primary progressive multiple sclerosis	T2LV	T2 lesion volume
pRNFL	peripapillary retinal nerve fibre layer	TE	echo time
PROM	patient-reported outcome measure	TMF	trial master file
PSIR	phase-sensitive inversion recovery	TR	repetition time
PWSAD	peak width of skeletonised axial diffusivity	TSC	Trial Steering Committee
PWSD	peak width of skeletonised diffusivity	UCL	University College London
PWSFA	peak width of skeletonised fractional anisotropy	VAS	visual analogue scale
PWSMD	peak width of skeletonised mean diffusivity	VOI	volume of interest

Plain English summary

Multiple sclerosis is a disabling and progressive neurological disease that affects approximately 120,000 people in the UK. Many people with multiple sclerosis experience two phases of disease called relapsing–remitting multiple sclerosis and secondary progressive multiple sclerosis. Relapsing–remitting multiple sclerosis is often characterised by periods of ‘attacks’ (relapses) interspersed with periods of ‘remission’ with no or few disease symptoms. The attacks are due to inflammation of the nerves and the insulation (called myelin) that surrounds the nerves. Secondary progressive multiple sclerosis, which ultimately affects most people with multiple sclerosis after 10–15 years from disease onset, results from nerve death (called neurodegeneration) and relentless disability. Unlike relapsing–remitting multiple sclerosis, there are few treatments with limited effects that can slow down the disability accrual in secondary progressive multiple sclerosis. MS-SMART (Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial) was a randomised and blinded trial that investigated three drugs (i.e. amiloride, fluoxetine and riluzole) that showed potential to prevent nerve death in multiple sclerosis. Randomisation means that participants can get any one of the three active drugs or the inactive placebo/dummy; blinded means that neither the participants nor the investigators will know which drug (or placebo) the participants are receiving. All participants in MS-SMART were planned to have brain magnetic resonance imaging scans before starting the trial and after 96 weeks, which were used to measure brain shrinkage – a normal process of ageing that occurs faster in people with multiple sclerosis and is thought to reflect nerve death (neurodegeneration). Across 13 UK clinical neuroscience centres, 445 people with secondary progressive multiple sclerosis were enrolled and each person was followed up for 96 weeks between December 2014 and July 2018. When we completed our analyses, we found no difference in the brain shrinkage rates between participants receiving amiloride, fluoxetine or riluzole and the dummy, suggesting that these drugs do not prevent nerve death (neurodegeneration). The results also suggest that testing three drugs simultaneously in one trial (rather than one by one) is feasible in secondary progressive multiple sclerosis.

Scientific summary

Background

Multiple sclerosis is an immune-mediated demyelinating disease of the central nervous system affecting approximately 120,000 people in the UK and 2.5 million people globally. Multiple sclerosis generally starts with a relapsing–remitting clinical course, characterised by relapses, that is, episodes of neurological dysfunction lasting at least 24 hours in the absence of infection, followed by various degrees of remission. After a mean of 10–15 years, most patients with relapsing–remitting multiple sclerosis enter into a phase characterised by gradual progression of disability, called secondary progressive multiple sclerosis.

There is no cure for multiple sclerosis, but there are drugs that can modify the clinical course of the disease in the early stages when the disease is defined as relapsing–remitting multiple sclerosis. However, these drugs have no substantial effect on stopping or slowing the relentless disability accrual in secondary progressive multiple sclerosis.

The underlying mechanisms related to secondary progression are complex and still unclear; however, it seems that progressive neuroaxonal loss or neurodegeneration plays the major role in the accumulation of irreversible disability. It is likely that several physiopathological processes, such as redistribution of sodium channels across the demyelinated axon, mitochondrial dysfunction and excitotoxicity, act in concert, culminating in intra-axonal calcium accumulation and irreversible structural damage of the axon. In animal models of multiple sclerosis, researchers have found that amiloride, fluoxetine and riluzole can prevent this axonal structural damage and, therefore, act as neuroprotective drugs. Findings from these pre-clinical studies were translated into clinical research by testing amiloride, fluoxetine and riluzole in small trials of patients with progressive multiple sclerosis, which showed promising preliminary results.

Objectives

The primary objective of the Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial (MS-SMART) was to establish whether or not any of the three selected drugs (i.e. amiloride, fluoxetine and riluzole) was able to decrease the progression of brain atrophy in people with secondary progressive multiple sclerosis over 96 weeks as assessed by magnetic resonance imaging-derived percentage brain volume change. The compounds chosen were targeted specifically to be axonal protective.

Secondary objectives included establishing whether or not a multiarm trial strategy was an efficient way of screening drugs in secondary progressive multiple sclerosis and could become a template for future studies, exploring any anti-inflammatory drug activity and examining the clinical and patient-reported effects of neuroprotection.

Exploratory objectives included assessing neuroprotection in the new multiple sclerosis lesions and in the cortex; evaluating myelination with magnetisation transfer ratio imaging and brain metabolite concentrations with magnetic resonance spectroscopy; estimating neuroprotection in the spinal cord; and evaluating neuroprotection with diffusion tensor imaging, optical coherence tomography and cerebrospinal fluid neurofilaments.

Methods

This was an investigator-led double-blind, placebo-controlled, randomised multiarm Phase IIb trial carried out at 13 UK clinical neuroscience centres. The trial was designed for patients with confirmed diagnosis of secondary progressive multiple sclerosis with evidence of steady progression (rather than relapses) as a major cause of increasing disability in the preceding 2 years. Eligible patients were aged 25 to 65 years (inclusive), were still able to walk at least 20 metres (Expanded Disability Status Scale score 4.0–6.5), were able to undergo magnetic resonance imaging scans and were not on disease-modifying drugs, immunosuppressants or selective serotonin reuptake inhibitors. Pregnant women and patients with the following comorbidities were excluded: depression, bipolar disorder, epilepsy, glaucoma, bleeding disorders or other significant diseases. After consenting and screening for eligibility, participants whose magnetic resonance imaging scans were judged to be suitable for primary outcome analysis were randomised in a 1 : 1 : 1 : 1 ratio to receive one of the three active drugs – amiloride (5 mg), fluoxetine (20 mg), riluzole (50 mg) – or placebo twice daily. After baseline, patients were assessed for safety at weeks 4, 8, 12, 24, 36, 48, 72 and 96. A wide range of clinician- and patient-reported outcome measures were collected yearly and included the Expanded Disability Status Scale, Timed-25-Foot Walk, 9-Hole Peg Test, Paced Auditory Serial Addition Test, Multiple Sclerosis Functional Composite, Symbol Digit Modalities Test, high-contrast visual acuity (100%), and Sloan Low Contrast Visual Acuity (5%, 2.5%, 1.25%), Multiple Sclerosis Impact Scale 29 items, version 2, Multiple Sclerosis Walking Scale, version 2, Neurological Fatigue Index and health-related quality of life (EuroQol-5 Dimensions, five-level version).

Magnetic resonance imaging was carried out at baseline, week 24 and week 96. Magnetic resonance imaging scans included brain volumetric sequences analysed using the Structural Image Evaluation using Normalisation of Atrophy method to obtain the percentage brain volume change after 96 weeks, which was the primary end point of the study. Secondary magnetic resonance imaging end points were count of new and enlarging T2 lesions, and percentage brain volume change at 24 weeks. Clinical secondary end points were changes over time in the clinical variables. At the London and Edinburgh sites, optional substudies were carried out that included an advanced magnetic resonance imaging protocol (at the London site this included magnetisation transfer ratio, magnetic resonance spectroscopy and spinal cord imaging; at the Edinburgh site, this included magnetisation transfer ratio, magnetic resonance spectroscopy and diffusion tensor imaging); cerebrospinal fluid (at the London site); and optical coherence tomography (at the London and Edinburgh sites). Exploratory end points included measures of central nervous system integrity or neuroprotection obtained with the substudies and the additional following measures: proportion of new and enlarging T2 lesions at 24 weeks being persistently T1 hypointense at 96 weeks; percentage grey matter volume change; predictive modelling of the primary and Expanded Disability Status Scale outcomes according to baseline magnetic resonance imaging/disability scores; and modelling of treatment effect according to baseline magnetic resonance imaging/disability scores.

No adjustment for multiplicity was made when analysing the secondary and exploratory end points. The interpretation of secondary and exploratory outcome analyses will be suitably cautious to reflect the high number of outcomes considered.

Results

A total of 547 participants were consented between December 2014 and June 2016. Four hundred and forty-five (81% of the total number screened) participants met all the eligibility criteria and were consecutively randomised to one of the three active treatments or placebo. The first randomisation occurred on 29 January 2015 and the last randomisation occurred on 22 June 2016. The last patient visit occurred on 4 July 2018. Participants were randomised to receive amiloride ($n = 111$), fluoxetine ($n = 111$), riluzole ($n = 111$) or placebo ($n = 112$). In total, 393 participants completed the study and

were analysed for the primary outcome (amiloride, $n = 99$; fluoxetine, $n = 96$; riluzole, $n = 99$; placebo, $n = 99$). Overall, 337 participants were adherent to allocated trial medication. Adherence was similar across treatment groups: amiloride, 83 out of 111 (75%); fluoxetine, 87 out of 111 (78%); riluzole, 84 out of 111 (76%); and placebo, 83 out of 112 (74%). Eighty-five participants permanently discontinued their assigned treatment after randomisation: amiloride 20 (18%), fluoxetine 24 (22%), riluzole 22 (20%) and placebo 19 (17%). Nineteen patients (4%) withdrew from the trial (three deceased, one on instruction from their treating clinician and 15 at the request of the participant), and a further 13 patients (3%) could not be contacted (recorded as lost to follow-up). Fifty-two patients (12%) did not attend the 96-week magnetic resonance imaging follow-up [amiloride 12 (11%), riluzole 12 (11%), fluoxetine 15 (14%) and placebo 13 (12%)].

In the course of the trial, unblinding occurred six times because of two deaths (one patient in the riluzole arm and one patient in the fluoxetine arm), three times because of serious adverse events requiring hospitalisation (two patients were on riluzole and one patient was on placebo) and one because of evidence of clinical worsening suspected to be due to study drugs (the patient was on fluoxetine).

Demographic characteristics were as follows: mean (standard deviation) age was 54.6 (7) years, number (proportion) of males was 147 (33%), the median (interquartile range) disease duration was 21 (15–29) years and Expanded Disability Status Scale score was 6.0 (5.0–6.5). Magnetic resonance imaging baseline characteristics were mean (standard deviation) brain volume 1422.6 ml (83.6 ml), median (interquartile range) T2 lesion volume 10.4 ml (4.1–18.6 ml).

No significant difference between any of the active arms and the placebo arm was seen with the primary outcome or percentage brain volume change at week 96. Amiloride minus placebo was 0.0% (Dunnett-adjusted 95% confidence interval –0.4% to 0.5%), fluoxetine minus placebo was –0.1% (Dunnett-adjusted 95% confidence interval –0.5% to 0.3%) and riluzole minus placebo was –0.1% (Dunnett-adjusted 95% confidence interval –0.6% to 0.3%).

Percentage brain volume change at 24 weeks was significantly lower in the fluoxetine arm than in the placebo arm (adjusted mean difference –0.31, 95% confidence interval –0.60 to –0.02; $p = 0.032$), but not for the other active treatment arms versus the placebo arm. There was no difference between any of the active treatment arms and placebo for percentage brain volume change between 24 and 96 weeks. No significant difference was detected in the number of new and enlarging T2 lesions at week 96 for amiloride and riluzole versus placebo. Patients treated with fluoxetine showed a significantly lower rate of new and enlarging T2 lesions than placebo.

There was no evidence of consistent or biologically plausible benefit over placebo on any of the clinical and patient-reported outcomes. Fifty-one patients (11%) experienced at least one relapse overall during follow-up.

There were no emergent safety issues in the four trial arms.

There were 244 patients originally consented to the advanced magnetic resonance imaging substudy, 308 originally consented to the optical coherence tomography substudy and 84 to the cerebrospinal fluid substudy. There were 206 patients randomised to the advanced magnetic resonance imaging substudy, 260 to the optical coherence tomography substudy and 70 to the cerebrospinal fluid substudy.

The adjusted mean differences between active drugs and placebo were not statistically significant. There was no significant difference in the proportion of new and enlarging T2 lesions at 24 weeks being persistently T1 hypointense at 96 weeks between the three active arms and placebo.

Considering the optical coherence tomography findings, no biologically plausible treatment effect was seen.

The cerebrospinal fluid study was small (overall 39 patients completed the study: 10 on amiloride, 11 on fluoxetine, nine on riluzole and nine on placebo), which limits the interpretation. The other cerebrospinal fluid biomarkers examined did not reveal any statistically significant differences after consideration of multiple testing.

Conclusions

MS-SMART demonstrates that a multiarm approach to an intractable neurodegenerative disease can be successful. This type of trial is efficient and has an appropriate patient burden.

The primary outcome performed as expected in the placebo arm. A large number of important secondary outcome data were measured. Novel mechanistic measures have given insight into the pathobiology of secondary progressive multiple sclerosis.

MS-SMART was well powered, the primary outcome progressed as expected in the placebo arm, blinding was robust, adherence was high and retention was high. Valuable information was obtained across the board for all secondary and exploratory measures, which will help to decide their place in future trial design as indicative and mechanistic measures.

The drug selection process underlying the choice of the three trial drugs also demonstrated successful proof of concept. Two of the shortlisted compounds (ibudilast and lipoic acid) showed positive phase 2 signals in other trials.

In summary, the MS-SMART approach has laid down the template for future Phase II drug testing in neurodegenerative disease. This will enable the research community to accelerate the testing of drugs in these very demanding situations, which have high health-care costs and burdens associated with them.

Recommendations for future research are:

1. Multiarm trial paradigms are efficient and feasible.
2. Systematic drug selection from both pre-clinical and Phase IIa data targeting axonal protection in secondary progressive multiple sclerosis is successful and should be updated.
3. In secondary progressive multiple sclerosis, whole-brain atrophy is a robust primary outcome, as shown by the occurrence of the expected increased brain atrophy in the placebo arm.

Trial registration

This trial is registered as ISRCTN28440672, NCT01910259 and EudraCT 2012-005394-31.

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Chapter 1 Introduction

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS) with a reported prevalence of between 1 in 500 and 1 in 1500 in Europe, North America and Australasia, and represents the commonest non-traumatic cause of disability affecting young adults in temperate latitudes.¹ Approximately 120,000 people in the UK and 2.5 million people globally have MS.² MS generally starts with a relapsing–remitting clinical course, characterised by relapses, that is episodes of neurological dysfunction lasting at least 24 hours in the absence of infection, followed by varying degrees of recovery or remission.³ After a mean of 10–15 years, most of the relapsing–remitting multiple sclerosis (RRMS) patients enter into a phase characterised by gradual progression of disability, called secondary progressive multiple sclerosis (SPMS). The underlying mechanisms of this secondary progression of MS are complex and still unclear; however, progressive neuroaxonal loss or neurodegeneration plays a major role in the accumulation of irreversible disability.^{4–12} People with SPMS may experience severe limitations as a result of symptoms affecting walking, balance, vision, cognition, pain control, and bladder and bowel function.⁷

The disease-modifying therapies (DMTs) available for RRMS have immunomodulatory and/or anti-inflammatory properties. They are effective in reducing relapse rate and the number of new lesions as detected by magnetic resonance imaging (MRI), and they can also delay the disability progression and the rate of conversion to SPMS. Unlike RRMS, for which an increasing number of DMTs have been being developed, and with the recent exception of siponimod (which has modest effectiveness¹³) there is a paucity of disease modification in SPMS.^{14,15}

Mechanistic processes underlying neurodegeneration in secondary progressive multiple sclerosis

In MS, the mechanisms of neurodegeneration may overlap with other conditions, and it is speculated that there are central neurodegenerative paths common to other neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and motor neuron disease. However, it should be highlighted that the pathology of SPMS has distinctive features: oligodendrocyte loss/demyelination, energy failure, glial (astrocyte/microglial)-mediated production of reactive oxygen/nitric oxide species, and excitotoxicity.¹⁶

In demyelinated plaques, the pathological hallmark of MS, axons lack trophic support mediated by oligodendrocytes and become vulnerable to injury or physiological stress. Furthermore, demyelinated axons show a significant redistribution of sodium channels, which were previously restricted to the nodes of Ranvier. This adaptive response requires neurons to spend more energy to sustain electrical transmission, leading to a circumstance known as 'energy failure'.^{17–19} This is the result of an imbalance between energy demand and energy production in the demyelinated axons and results in an increased intracellular calcium influx, which is toxic to the axon. Axonal energy production is faulty as a result of mitochondrial damage mostly triggered by the production of reactive oxygen and nitric oxide species by activated microglia and macrophages.^{20,21} In MS-derived tissue samples and in experimental models of MS, lactic acidosis due to energy failure leads to the activation of a class of acid-sensing ion channels (ASICs) on neurons and oligodendrocytes.^{22,23} This represents an additional mechanism whereby intra-axonal cation excess leads to neuroaxonal damage. In addition, glutamate release by activated inflammatory cells, such as microglia, activates extra-synaptic N-methyl-D-aspartate (NMDA) receptors, which are cation channels permeable to calcium. The overstimulation of NMDA receptor-activated channels, known as excitotoxicity, is another mechanism implicated in the increased intracellular calcium concentration in neurons.^{24,25}

In summary, it is clear that the maladaptive ionic response to demyelination observed in MS sets in train a negative cycle of energy failure and linked processes including reactive oxygen species production by glia, mitochondrial inhibition and ASIC upregulation that converge around a common process of intra-axonal cation excess that initiates secondary calcium-mediated injury and, ultimately, death cascades including excitotoxicity.

Rationale for study and drug selection

Against the background of the neurodegenerative processes outlined above, we prospectively sought to identify existing putative neuroprotective drugs that target these pivotal neurodegeneration-causing pathways as the most biologically plausible approach to slowing disease progression in SPMS.

We followed a systematic approach to drug identification that includes drug rescue and repurposing, to shorten the time from target selection to regulatory approval. Drug rescue (drugs at an advanced stage of development but abandoned before approval) and repurposing (already approved drugs) exploit existing clinical efficacy, safety and regulatory data, and represent an interesting approach to reduce both cost and time in the process of drug licencing.^{26,27} For this reason, we first undertook an MS-Clinical Trials Network commissioned systematic review of animal and human trials of putative neuroprotective drugs.^{28,29} For the human analysis, given the existence of common pathways in neurodegeneration, we did not limit the research to MS, but also included amyotrophic lateral sclerosis, Huntington's disease, Alzheimer's disease and Parkinson's disease. Searching PubMed, EMBASE, the Institute for Scientific Information ($n = 27,000$) and the Cochrane Group MS database ($n = 2600$) returned 120 drugs for which summaries were generated that included analysis of mechanism of action, scores for safety, study quality, efficacy and sample size. Two clinicians then independently reviewed the 120 drugs and excluded 68 as unsuitable using predefined criteria. A specially convened International MS Drug Selection meeting comprising expert representation from the Cochrane MS group, neuroscientists (including those from the US National Institutes of Health), neurologists, brain imaging experts, people with MS, trial methodologists and industry considered the remaining 52 drugs. Putting an emphasis on clinical efficacy, structured discussions over three rounds of detailed scrutiny identified seven drugs (ibudilast, riluzole, amiloride, pirfenidone, fluoxetine, oxcarbamazepine and polyunsaturated fatty acids class dietary supplements) that were further ranked and grouped against class of action/mechanistic plausibility noting that we were looking for drugs that target the pivotal neurodegenerative pathways discussed above. From those seven drugs, ibudilast, riluzole and amiloride were the three drugs originally chosen, but because of drug supply issues, fluoxetine replaced ibudilast.²⁸

MS-SMART tested the efficacy and mechanism of action of these three repurposed drugs (amiloride, fluoxetine and riluzole) in people with SPMS. MS-SMART was a Type B trial, as the investigational medicinal products were all in human use, had a good safety profile but were not used for this patient population. Critically for the purpose of MS-SMART, all three drugs had shown promise in early-phase human MS clinical trials and targeted one or more of the pivotal neurodegenerative-causing pathways implicated in SPMS.²⁹

Amiloride

Amiloride is a widely used diuretic and antagonises ASICs. This last mode of action is thought to be responsible for the myeloprotective and neuroprotective effects in both human and experimental models of progressive MS. Seventeen patients affected by primary progressive multiple sclerosis (PPMS) were enrolled in a Phase IIa trial, divided into a pre-treatment phase (1 year) and an amiloride treatment phase (1 year), at 5 mg bis in die (b.i.d.). During the amiloride treatment phase, patients showed a significant reduction in the whole-brain atrophy rate compared to pre treatment ($p = 0.009$). Also, corpus callosal radial diffusivity (RD) (myelin integrity) and thalamic mean diffusivity (MD) (structural integrity) showed a significant improvement.³⁰

Fluoxetine

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that is widely used to treat depression. The main properties of this drug relevant for SPMS were the stimulation of glycogenolysis and the induction of production of brain-derived neurotrophic factor in rodent astrocyte cultures. A significantly improved cerebral white matter *N*-acetylaspartate (NAA)/creatinine ratio was found on MRI, suggesting that the drug may improve axonal mitochondrial energy metabolism. Another possible mechanism of action of this drug is the suppression of the antigen-presenting capacity of glial cells.^{31,32} Moreover, SSRIs have been demonstrated to ameliorate measures of dependence in patients with stroke. Two Phase IIa trials already tested fluoxetine in MS. In a cohort of 40 MS patients (10% SPMS) there was a significant reduction in relapse rate incidence to 0.54 (95% CI 0.29 to 0.98) with a trend towards reduction in new inflammatory lesions.³³ In the second trial, 42 patients with SPMS/PPMS (SPMS, *n* = 69%) were randomised in a 1 : 1 ratio to 40 mg or placebo over 2 years.³⁴ No statistical differences were seen in overall progression or in grey or white matter volume changes, but there were trends in favour of fluoxetine in relation to disability progression. This second trial was terminated early because of drug expiry issues and was considered to be underpowered.

Riluzole

Riluzole is a drug already licensed for motor neuron disease/amyotrophic lateral sclerosis and has two modes of action that may target neurodegeneration in SPMS: reducing glutamate release and antagonism of voltage-dependent sodium channels.³⁵ Sixteen patients with progressive MS were enrolled in a Phase IIa trial and studied for 1 year before treatment, followed by riluzole 50 mg b.i.d. for 1 year.³⁶ The primary outcome was the change in cervical spinal cord cross-sectional area, which showed a reduction from -2% (year 1) to -0.2% (year 2). Moreover, the increase in T1 hypointense lesion load was reduced from 15% in year 1 to 6% in year 2 and there was a reduction in brain parenchymal fraction of -1.0% (year 1) and -0.7% (year 2).

Paraclinical biomarkers of neurodegeneration

Magnetic resonance imaging

Magnetic resonance imaging is central to the diagnosis of MS as well as to guide and monitor treatment response. MRI-derived metrics have been extensively used as an interim outcome measure for randomised clinical trials in MS. In RRMS, quantification of new gadolinium-enhancing lesions or T2-weighted lesions from MRI is generally used as the primary outcome measure at phase 2 to test the efficacy of new DMTs in development.³⁷⁻³⁹ In SPMS, although there may be some effect on new lesion count and lesion volume, the main MRI parameter for investigating neurodegeneration is the change (reduction) in brain volume (BV). Compared with age-matched healthy controls, there is a greater decrease in BV over time in SPMS, termed atrophy rate, which can be quantified by MRI. On average there is 0.5–1% loss of BV per year in SPMS. MRI studies have demonstrated a correlation between BV loss and disability in SPMS. A decrease in BV is seen at all stages of MS, and especially in SPMS. Neuroaxonal tissue constitutes a large proportion of BV and the increased rate of brain atrophy has been interpreted as evidence for neuroaxonal loss. Brain atrophy is correlated with disability and cognitive impairment in MS.⁴⁰⁻⁴⁹

A large variety of advanced MRI techniques have been investigated in MS to both quantify and clarify neurodegeneration. In MS-SMART, other than BV and new and enlarging T2 lesion measures, several other MRI technologies were explored and are now described.

Proton magnetic resonance spectroscopy (MRS) was used to measure brain metabolites including glutamate, NAA and myoinositol.⁵⁰⁻⁵² High levels of glutamate, an excitatory neurotransmitter, are responsible for neuronal excitotoxicity, which was specifically targeted by riluzole as a proposed mechanism of action. NAA, a metabolite synthesised in mitochondria, is associated with axonal integrity and mitochondrial function and is used as a marker of neuroprotection and energy metabolism.

Myoinositol is considered a marker of glial cell proliferation and activation (astrocytes and microglia), which are prominent features of CNS inflammation and contribute to neurodegeneration in SPMS.

Magnetisation transfer ratio (MTR) is a technique that strongly reflects the amount of myelin in the brain, but can also be influenced by inflammation and axonal density. There is an exchange of magnetisation between protons that are freely mobile and those that are bound to macromolecules. The extent of this exchange provides an indication of the amount of macromolecular structure in tissue and can be measured with the MTR.^{53,54} Myelin has a major effect on MTR and in MS lower MTR is seen in demyelinated rather than remyelinated white matter lesions. The amount of signal decrease is thought to suggest damage to myelin or to the axonal membranes. The MTR measure provides an indication of the extent of axonal loss associated with new inflammatory-demyelinating white matter lesions. MTR has provided many insights regarding the evolution of demyelination and remyelination and was used in MS-SMART to measure the potential benefit on remyelination and putative neuroprotective therapy effects.⁵⁵

Diffusion tensor imaging (DTI) can quantitatively detect brain microstructural changes by calculating parameters, such as MD, which is determined by the overall water motion, and fractional anisotropy (FA), which reflects the uniformity of the direction of the diffusion of water molecules. DTI measures may be considered as markers of demyelination and axonal loss.⁵⁶ Strong diffusion-encoding gradients allow measurement of water diffusivity in the direction parallel to white matter fibres and perpendicular to this, and changes in these correlate with axonal damage and demyelination. FA derived from the diffusion tensor demonstrates the tendency of water molecules to diffuse in one direction and is a further marker of white matter integrity and myelination status. Abnormal DTI values have been demonstrated in both white matter lesions and normal-appearing white matter (NAWM) in MS, and further deterioration in the placebo arm is expected over the duration of the trial. Stabilisation of these values in treated arms of the study may indicate prevention of further tissue loss within white matter tracts.

In MS, both the brain and the spinal cord are affected. Indeed, up to 90% of patients with MS develop spinal cord focal lesions or diffuse abnormalities, which contribute to disability. Neuronal loss in the cord may occur for direct damage owing to white and grey matter (GM) demyelination or to secondary Wallerian degeneration.^{57–61} These phenomena can be captured by quantification of spinal cord atrophy, an aspect that can be substantial in progressive MS.⁶² The most common method for assessing in vivo spinal cord atrophy is to measure the cross-sectional area at specific anatomical levels, typically in the cervical region in the area from C2 to C5, using MRI. Image acquisition and segmentation of the upper cervical region are more efficient than in other parts of the cord from a technical perspective,⁶³ and are thought to be more representative of the neurodegenerative process in the cord as lesions are more common in the cervical tract⁶⁴ and this cord section may reflect destructive processes lower down in the cord via Wallerian degeneration. There is a strong clinical–radiological association between spinal cord atrophy and disability measures in MS, particularly in the progressive stages of the disease.^{62,65–67} In a recent systematic review and meta-analysis (including 22 longitudinal studies and > 1000 patients), a mean upper cervical cord cross-sectional area of 73.07 mm² in all MS patients and 68.55 mm² in SPMS patients (vs. 80.87 mm² in healthy controls⁶⁸) was found, with a rate of spinal cord atrophy of 1.78% per year (95% CI 1.28% to 2.27% per year), potentially supporting the use of MRI-derived cervical cord area measurement as an outcome measure of neurodegeneration in MS trials.

Optical coherence tomography

The anterior visual pathway offers the possibility to study in vivo neuroaxonal loss in MS. Since the pioneering studies of Frisén and Hoyt,⁶⁹ who described qualitative changes in the retinal nerve fibre layer (RNFL) in patients with MS, and Frohman,⁷⁰ who found that about 50% of ganglion cells must be lost before focal RNFL defects are detectable, many attempts have been made to improve our ability to visualise the neuronal retinal layers. Optical coherence tomography (OCT) is an increasingly

recognised, non-invasive imaging tool that allows investigation of the architecture of the neural retina, which had been virtually inaccessible until the advent of high-resolution spectral domain optical coherence tomography and has fundamentally changed our ability to qualitatively and quantitatively assess the eye.⁷¹

Optical coherence tomography relies on interferometry of near-infrared light to construct very high-resolution images of the retinal layers. The most visible layer is the RNFL, comprising unmyelinated axons in a supportive connective tissue framework. The RNFL axons originate from retinal ganglion cell bodies, and continue through the optic nerve, chiasm and tract (where they are myelinated), to synapses in the lateral geniculate bodies. In acute optic neuritis, RNFL thickness increases because of optic nerve swelling. Subsequently, its thickness reduces, indicating significant axonal loss.⁷² The optic nerve lesion leads to retrograde degeneration of the RNFL, a relatively pure compartment of unmyelinated axons whose thickness can be measured sensitively and non-invasively using OCT. Therefore, quantification of peripapillary retinal nerve fibre layer (pRNFL) thickness provides a plausible biomarker of axonal loss.⁷³ In addition, abnormalities of the retinal layers other than the pRNFL have been observed in post-mortem specimens from patients with MS, in which 79% of eyes exhibited ganglion cell loss and 40% showed amacrine and bipolar cell loss in the inner nuclear layer. These findings have been corroborated in vivo by OCT, demonstrating thinning of the ganglion cell layer (GCL) and inner plexiform layer (IPL), and are associated with reductions in visual function and vision-specific quality of life.⁷⁴

Many studies have reported an inverse correlation between pRNFL thickness and Expanded Disability Status Scale (EDSS) score in MS, and the strongest correlation was found for patients not affected by optic neuritis.⁷⁵ A recent meta-analysis examining studies using OCT in mixed cohorts of MS patients confirmed that, when compared with healthy controls, pRNFL and the complex GCL and IPL were decreased in both MS optic neuritis and non-optic neuritis eyes.⁷⁶ One study found that patients with MS and a pRNFL thickness of up to 87 μm (as measured with a Cirrus OCT machine; Carl Zeiss, Dublin, CA, USA) or 88 μm (as measured with a Spectralis OCT machine; Heidelberg Engineering, Heidelberg, Germany) had roughly twice the risk of disability worsening during follow-up compared with patients with thicker pRNFL.⁷⁷ This risk was independent of other factors known to be associated with disability worsening, including age, disease duration, baseline level of disability (EDSS) and the use of DMTs. It was suggested that the process of neurodegeneration within the retina could reflect similar processes occurring more diffusely in the brain and spinal cord. An extensive review of the literature confirmed that using OCT in MS can provide valid, reliable and reproducible data to track the process of neurodegeneration within the retina of patients with MS with optic neuropathy. pRNFL thickness and macular volume analyses could serve as a surrogate biomarker and primary outcome measure to confirm the neuroprotective effects of new drugs.⁷⁰

Optical coherence tomography has good analytical reproducibility, is cost-effective, correlates with clinical measures (loss of visual function, cognitive decline) and is predictive of a clinical outcome (poor visual recovery).⁷⁵ Finally, atrophy of the complex GCL and IPL appears to mirror whole-brain (and particularly GM) atrophy, especially in progressive MS, supporting the use of OCT in clinical trials.⁷⁸

Despite the evidence described above, and although serial OCT-measured pRNFL thickness has been proposed as a measure of neurodegeneration for clinical trials in MS, longitudinal observations are largely confined to RRMS, underscoring the need to develop the data set in progressive MS.^{76,77}

Cerebrospinal fluid

Cerebrospinal fluid (CSF) measures are potential outcome measures in SPMS trials.

Neurofilaments represent a component of the mature cytoskeleton of neurons,⁷⁹ and are composed of neurofilament light (NfL), neurofilament medium (NfM) and neurofilament heavy (NfH) chain subunits. All the pathological processes that cause neuroaxonal damage release neurofilament proteins into the

extracellular space, CSF and, depending on the extent of damage, the peripheral blood; therefore, CSF and blood neurofilament levels are considered as markers of neuroaxonal damage.

Disease-modifying therapies for RRMS, in particular the highly active therapies, have all demonstrated reduction in CSF NfL levels (fingolimod,⁸⁰ natalizumab,⁸¹ ocrelizumab⁸²), indicating a relationship between anti-inflammatory therapies and neuronal damage resolution. In SPMS, serum NfH and CSF NfH in a cohort of patients compliant with lamotrigine (a putative neuroprotectant), demonstrated lower levels than placebo.⁸³ This emphasises the mechanistic utility of sampling the CSF for such markers and exploring neurofilament and other measures in an established SPMS cohort.

Study objectives

The main aim of MS-SMART was to determine the efficacy of three likely neuroprotective agents, in a multiarm approach, to increase biological understanding of the disease process and to further our knowledge of interim markers of neurological damage. This was undertaken using MRI, disability measurements, OCT and targeted CSF analysis.

Primary objective

The primary objective of the MS-SMART study was to establish whether or not any of the three selected drugs (i.e. amiloride, fluoxetine and riluzole) were able to decrease the progression of BV loss in people with SPMS over 96 weeks, as assessed by MRI-derived percentage brain volume change (PBVC).

Secondary objectives

The secondary objectives were to:

1. establish that a multiarm trial strategy was an efficient way of screening drugs in SPMS and can become the template for future work
2. explore any anti-inflammatory drug activity (measured by counting the new and enlarging T2-weighted white matter lesions)
3. examine for evidence of pseudo-atrophy by MRI
4. examine the clinical effect of neuroprotection as measured by clinician- and patient-reported outcome measures (PROMs)
5. collect basic health-related quality-of-life data.

Exploratory objectives

The exploratory objectives were to:

1. assess neuroprotection in new lesions by estimating persistent new T1-weighted hypointense lesion (or 'black holes') count
2. assess cortical neuroprotection by evaluation of GM volume change
3. evaluate myelination using MTR
4. assess spinal cord neuroprotection using MRI
5. quantify neuronal mitochondrial dysfunction, prevention of glial cell inflammation and prevention of excitotoxicity as measured by MRS metabolites
6. quantify neuroprotection using DTI as an index of white matter integrity
7. evaluate neuroprotection using OCT measures
8. quantify neuroprotection using CSF neurofilaments
9. investigate treatment effect and predictive models of brain atrophy (by MRI) and clinical disability (by EDSS) at 96 weeks using baseline MRI and disability scores.

Patient and public involvement

The scientific approach in MS-SMART was recognised as being completely in tune with the wishes of people with SPMS.

Patient and public involvement (PPI) has been central to the genesis of this study. The UK MS Society has a long and active history of PPI. This was realised in the formation of the MS Society Clinical Trials Network. In the MS-SMART study this was evidenced by strong and repeated PPI sessions in the design and conduct of the study, specifically:

1) the Group emphasised the importance of working with early SPMS patients (EDSS scores below 6.5) prior to the ambulatory phase in light that any neuroprotective drug is likely to have most benefit; 2) a multi-arm design was preferable to standard single arm versus placebo to ensure that a maximum number of patients would have access to putative neuroprotective repurposed drugs compared to placebo; 3) they considered that the burden of advanced MRI protocol was acceptable to patients in view of the potential advancement that will come from the imaging analysis to the mechanistic elucidation.

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All PPI recommendations were taken on board. One of the principal investigators (RB) was a person with progressive MS. The final study results were communicated in a specially developed video,⁸⁴ in conjunction with the UK MS Society, to all trial participants as the trial results were announced. Participants were also told of their treatment allocation if they so wished.

Chapter 2 Methods

Trial design

MS-SMART was an investigator-led, multicentre, multiarm, double-blind, placebo-controlled, parallel-group, randomised Phase IIb trial that compared three putative neuroprotective therapies (i.e. amiloride, fluoxetine and riluzole) versus a shared placebo arm in people with SPMS. Participants were allocated 1 : 1 : 1 : 1 to active treatment with amiloride, fluoxetine or riluzole or a matching placebo. The duration of the trial for participants was 96 weeks.

Setting

MS-SMART took place at 13 UK clinical neuroscience centres in London, Edinburgh, Liverpool, Sheffield, Brighton, Truro, Oxford, Stoke-on-Trent, Plymouth, Newcastle, Leeds, Nottingham and Glasgow. The two main trial sites were University College London (UCL) Queen Square Institute of Neurology in London (hereafter called London) and the University of Edinburgh Anne Rowling Regenerative Neurology Clinic in Edinburgh (hereafter called Edinburgh).

Substudies

At the London and/or Edinburgh sites, the following optional substudies were in place:

- the advanced MRI substudy (at both the London and Edinburgh sites, but with different imaging protocols)
- CSF (only at the London site)
- OCT (at both the London and the Edinburgh sites).

Study approvals

MS-SMART was approved by the Scotland A Research Ethics Committee (REC) on 13 January 2013 (REC reference: 13/SS/0007). The trial was assigned ClinicalTrials.gov identifier (NCT number) NCT01910259;2012-005394-3; and the International Standard Randomised Controlled Trial Number (ISRCTN) ISRCTN28440672.

Participants

Participants were aged 25–65 years, had a diagnosis of SPMS with evidence of disease progression independent of relapses in the previous 2 years and had an EDSS score of 4.0–6.5 at baseline.

Inclusion criteria

- Confirmed diagnosis of SPMS. Steady progression rather than relapse must be the major cause of increasing disability in the preceding 2 years. Progression can be evident from either an increase of at least 1 point in EDSS score or clinical documentation of increasing disability in the patient's notes.
- EDSS score of 4.0–6.5.
- Aged 25–65 years, inclusive.

- Women and men with partners of childbearing potential had to use an appropriate method of contraception.
- Women must have a negative pregnancy test within 7 days prior to the baseline.
- Willing and able to comply with the trial protocol (e.g. can tolerate MRI and fulfils the requirements for MRI).
- Give written informed consent.

Exclusion criteria

- Pregnant or breastfeeding females.
- Baseline MRI scan not of adequate quality for analysis.
- Significant organ comorbidity.
- Relapse within 3 months of baseline visit.
- Intravenous or oral steroid treatment for a MS relapse/progression within 3 months of baseline visit.
- Use of simvastatin at 80-mg dose within 3 months of baseline visit.
- Commencement of fampridine within 6 months of baseline visit.
- Use of immunosuppressants (e.g. azathioprine, methotrexate, ciclosporine) or first-generation disease-modifying therapies (β -interferons, glatiramer acetate) within 6 months of baseline visit.
- Use of mitoxantrone, natalizumab, alemtuzumab, daclizumab, fingolimod, fumarate, teriflunomide, laquinomod or other experimental disease-modifying therapy within 12 months of baseline visit.
- PPMS.
- RRMS.
- Known hypersensitivity to the active substances and their excipients to any of the active drugs for this trial.
- Use of a SSRI within 6 months of the baseline visit.
- Current use of tamoxifen.
- Current use of herbal treatments containing St. John's wort.
- History of bleeding disorders or current use of anticoagulants.
- Use of monoamine oxidase inhibitors, phenytoin, L-tryptophan and/or neuroleptic drugs, lithium, chlorpropamide, triamterene or spironolactone within 6 months of the baseline visit.
- Current use of potassium supplements.
- Significant signs of depression and a Beck Depression Inventory, version 2 (BDI-II), score of ≥ 19 .
- Bipolar disorder.
- Epilepsy/seizures.
- Receiving or previously received electroconvulsive therapy treatment.
- Glaucoma.
- Routine screening blood values: liver function tests $> 3 \times$ upper limit of normal of site reference ranges (aspartate aminotransferase, alanine aminotransferase, bilirubin, gamma-glutamyl transferase); potassium levels of < 2.8 mmol/l or > 5.5 mmol/l; sodium levels of < 125 mmol/l, creatinine levels of > 13 mmol/l; a white blood cell count of $< 3 \times 10^9$ /l; lymphocytes count of $< 0.8 \times 10^9$ /l; a neutrophil count of $< 1.0 \times 10^9$ /l; a platelet count of $< 90 \times 10^9$ /l; and haemoglobin levels of < 80 g/l.

Recruitment of participants

The identification of the potential MS-SMART participants was conducted through several routes:

- in clinics run by principal investigators/neurologists, at participating hospital and clinic sites
- using existing MS research and other neurological databases, such as the Scottish Health Research Register (SHARE) (URL: www.registerforshare.org), containing contact details of people who previously consented to be contacted about research
- by primary care referrals
- by participants' self-referral through a dedicated MS-SMART website.

Pre-screening routes to check patients' eligibility were allowed:

- in-clinic briefing of potential participants about the study directly by a member of the clinical team
- initial telephone contact from a member of the research team to explain the trial.

All the contacted potential participants were provided with a study patient information leaflet (PIL) and then at an interval no less than 24 hours after receiving the PIL they were re-contacted to determine if they wanted to proceed to face-to-face screening. If required, with permission, the participant's general practitioner (GP)/neurologist was also contacted to provide written evidence that the patient met all the relevant eligibility criteria, before the screening visit in order to avoid unnecessary visits.

Following the pre-screening review at the London and Edinburgh sites, the potential participants were also provided with PILs for the optional relevant substudies.

The anonymity of all ineligible patients and all eligible patients who declined participation was maintained in every recruiting site. Anonymised information was collected including age, sex, date of screening, reason not eligible to participate (if applicable), reason for declining participation despite eligibility (if applicable) and any other reason for non-participation (if applicable).

Trial interventions

Investigational medicinal products

The intervention was a random allocation to amiloride (5 mg twice daily), or fluoxetine (20 mg twice daily), or riluzole (50 mg twice daily), or placebo (one capsule twice daily). For the first 4 weeks, participants were asked to take only one capsule daily (*Figure 1*). Details on dose modification and stopping rules are reported elsewhere.²⁹

The MS-SMART placebo comprised a size 00 capsule identical to the overencapsulated fluoxetine/amiloride/riluzole.

To evaluate the adherence to study drug, each participant was requested to bring back the unused study drug at each study visit and was asked about adherence. Participants had to record the number of capsules taken by using a diary card and to indicate any reason for non-adherence.

Screening visit

After the pre-screening phase, and at least 24 hours after receiving the PIL, patients underwent a formal face-to-face screening visit that involved documentation of written informed consent, review of medical history, concomitant medications, assessment of EDSS and BDI-II score, full physical clinical examination, recording of vital signs and collection of blood tests. After the screening phase, patients had a baseline brain MRI. In the majority of the recruitment sites, it was possible to perform the baseline MRI scan on the same day as the screening visit, decreasing travel costs and time commitment. A standard mandatory MRI protocol (defined as core MRI) was performed for all participants recruited in all sites. The London and Edinburgh sites performed optional MRI scan protocols (defined as advanced MRI), which included brain MTR, spectroscopy and DTI scans at the Edinburgh site, and brain MTR, spectroscopy and upper cervical cord at the UCL site. Each site subsequently transferred all scans to the Queen Square MS Centre Trial Office for central quality control and approval. Upon acceptance of the core MRI scans, the participants were then invited to take part to the baseline visit. At the London site, participants were also invited to take part in the optional OCT and CSF substudies. At the Edinburgh site, participants were

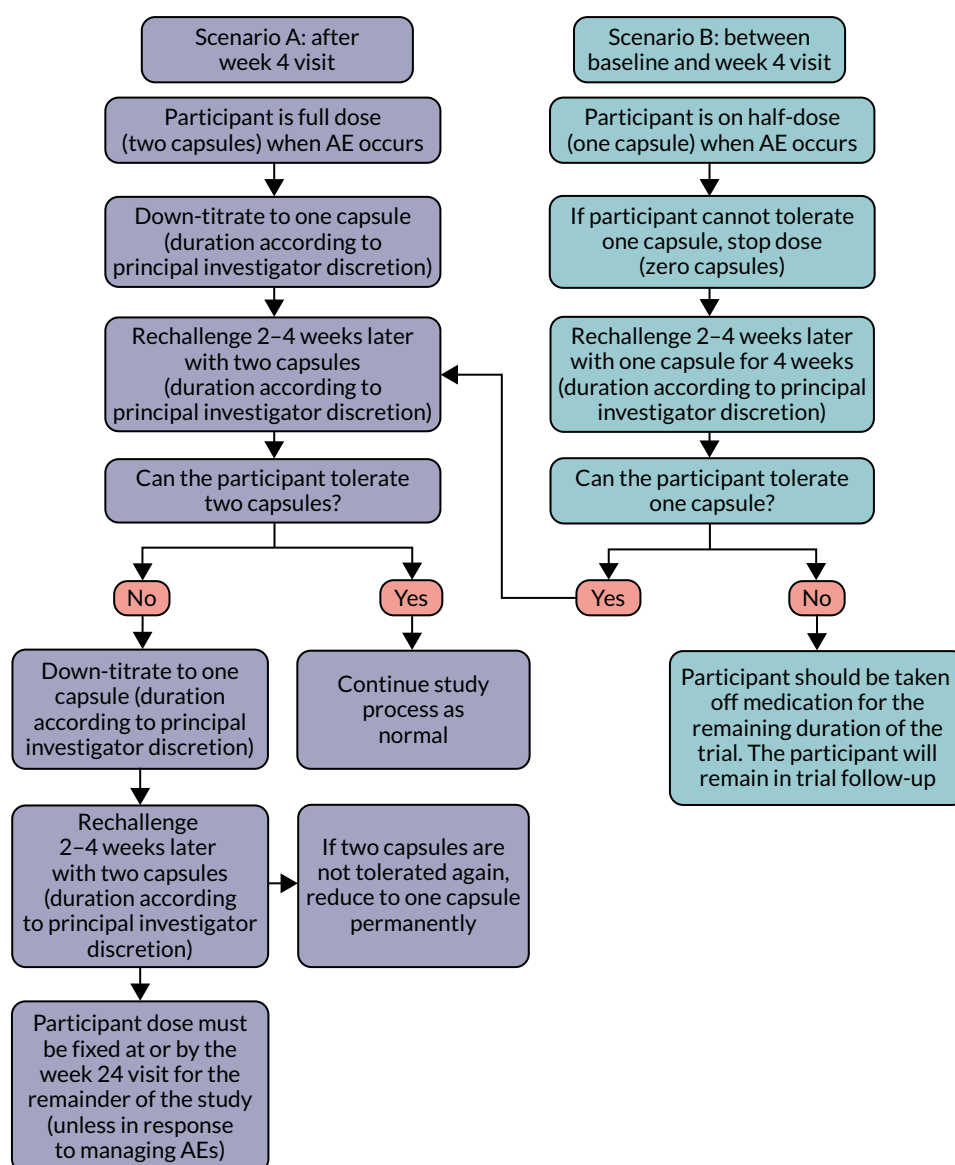


FIGURE 1 Drug titration scheme. AE, adverse event. Adapted from Connick *et al.*²⁹ This is an Open Access article distributed in accordance with the terms of the Creative Commons Attribution (CC BY 4.0) license, which permits others to distribute, remix, adapt and build upon this work, for commercial use, provided the original work is properly cited. See: <http://creativecommons.org/licenses/by/4.0/>.

invited to take part in the optional OCT substudy. *Figure 2* shows the substudies carried out at the London and Edinburgh sites.

Baseline visit

The activities at the baseline visit included detailed neurological evaluation, randomisation and collection of participant self-reported questionnaires. Rejection of the advanced MRI protocol only did not impede the enrolment of patients and, whenever possible, the rejected advanced MRI scans were repeated at the baseline visit. After completion of the baseline visit and randomisation, the study drugs, emergency card and patient diary were given to the participant.

For patients who consented to the substudies, OCT and lumbar puncture were also performed.

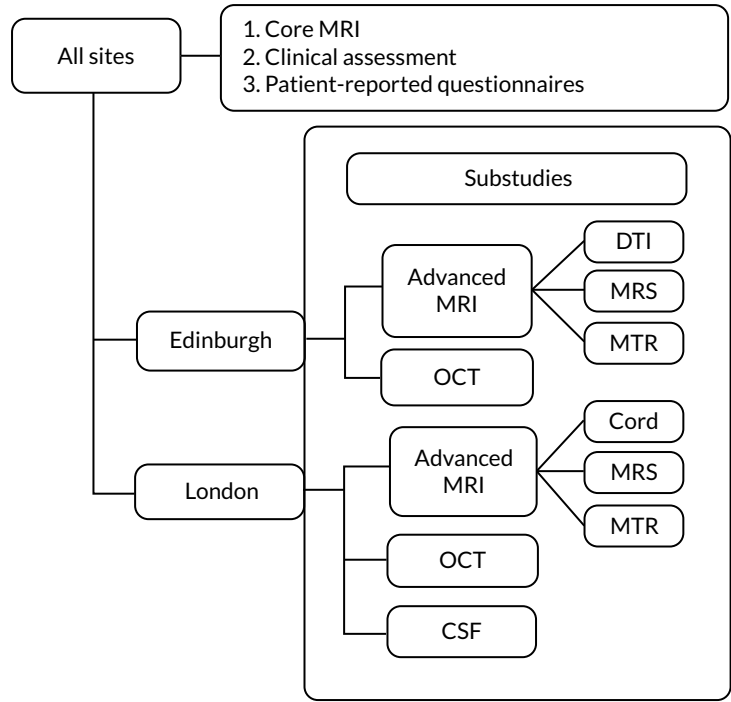


FIGURE 2 Diagrammatic representation of the substudies.

Follow-up visits

After the baseline visit, participants were reviewed at weeks 4, 8, 12, 24, 36, 48, 72 and 96 (Figure 3). At the week 48 and week 96 visits, a full neurological assessment was repeated and patients completed self-reported questionnaires. The MRI scan, instead, was repeated at week 24 and week 96. Standard clinical laboratory blood tests (chemistry and haematology) were performed for safety monitoring at each visit. Finally, at week 100, a safety telephone call was performed.

For patients taking part in the substudies, lumbar puncture was repeated at weeks 48 and 96, and OCT was repeated at week 96.

Patient flow

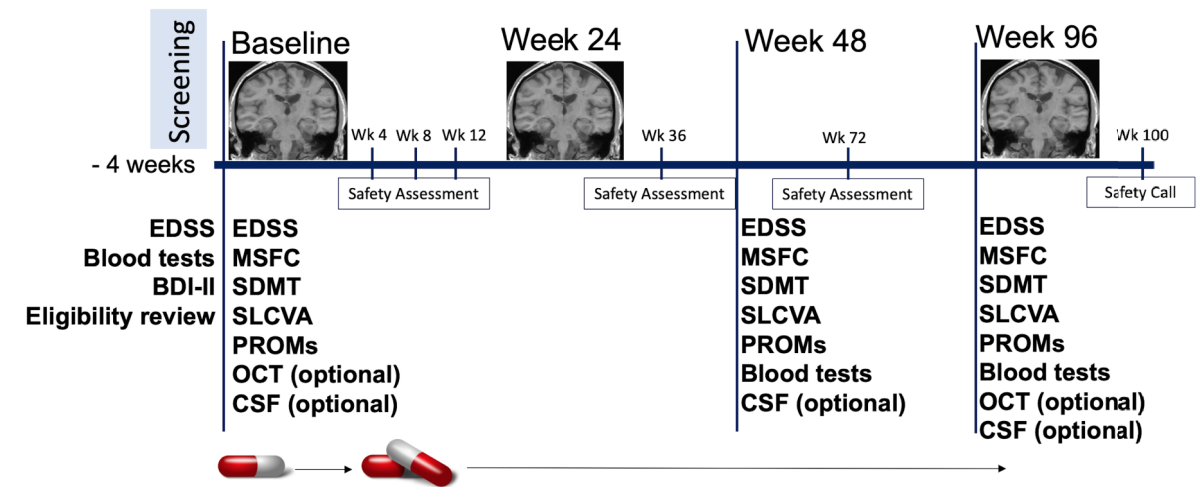


FIGURE 3 Patient visits. MSFC, multiple sclerosis functional composite; SDMT, Symbol Digit Modalities Test; SLCVA, Sloan Low Contrast Visual Acuity.

At each study site, there were treating physicians [responsible for dose adjustment and adverse event (AE) monitoring], research nurses and trained NeuroStatus-certified assessing physicians masked to the patients' history, who performed baseline and yearly clinical outcome assessments. Follow-up assessments were undertaken preferably by the same assessing physician to improve consistency.

Outcomes

Primary end point

Percentage brain volume change measured at 96 weeks using the Structural Image Evaluation using Normalization of Atrophy (SIENA) technique was chosen as the primary outcome measure in this trial. SIENA is an automated method that registers the follow-up scan to the baseline scan and produces an integral of the edge motion existing in each voxel in both scans. It then directly calculates the PBVC from those values.^{47,85} PBVC is considered as a marker of neurodegeneration.⁴⁸

Secondary end points

Secondary MRI end points were count of new and enlarging T2 lesions at 96 weeks and PBVC at 24 weeks (to estimate pseudoatrophy). Although new and enlarging T2 lesions appear to be less relevant than brain atrophy as a measure of neuroprotection in SPMS, they were included as a core outcome measure in order to detect an unanticipated immunomodulatory effect.

Clinical secondary end points were EDSS, Timed-25-Foot Walk (T25FW), 9-Hole Peg Test (9HPT), Paced Auditory Serial Addition Test (PASAT), Multiple Sclerosis Functional Composite (MSFC), Symbol Digit Modalities Test (SDMT), high-contrast visual acuity (HCVA) (100%), and Sloan Low Contrast Visual Acuity (SLCVA) (5%, 2.5%, 1.25%) obtained at baseline, 48 and 96 weeks. Time to first relapse was recorded. Patient-reported outcomes of the following were also measured at baseline and at 48 and 96 weeks: Multiple Sclerosis Impact Scale 29 items, version 2 (MSIS29v2), Multiple Sclerosis Walking Scale, version 2 (MSWSv2), Neurological Fatigue Index (NFI), Numeric Pain Rating Scale (NPRS), Brief Pain Inventory (BPI), Neuropathic Pain Scale (NPS) and health-related quality-of-life [EuroQol-5 Dimensions, five-level version (EQ-5D-5L)]. The MSFC z-score was normalised using participants' baseline scores.

Exploratory end points

Exploratory MRI end points were as follows:

- Proportion of new and enlarging T2 lesions at 24 weeks being persistently T1 hypointense at 96 weeks. Persistently T1 hypointense lesions exhibit greater axonal loss and could be a measure of neuroprotection. This end point was collected by all trial sites as part of the core MRI protocol.
- Percentage GM volume change (to assess cortical neuroprotection) at 96 weeks. GM atrophy is abundant in SPMS and shows robust correlations with disability. GM atrophy was investigated as an additional measure of neuroprotection. This end point was collected by all trial sites as part of the core MRI protocol.
- MR spectroscopy metabolite concentration including measures of NAA (reversal of neuroaxonal mitochondrial dysfunction), myoinositol (prevention of glial cell inflammation) and glutamate (prevention of excitotoxicity) at baseline and at 96 weeks. MR spectroscopy was part of the advanced MRI substudy and was performed at the London and Edinburgh sites.
- MTR in the GM and in the new lesions to determine demyelination and remyelination and MTR of NAWM to investigate tissue integrity. It was measured at baseline and at 96 weeks. MTR was part of the optional advanced MRI substudy and was performed at the London and Edinburgh sites.
- Upper cervical cord cross-sectional area (to investigate cord atrophy) measured on cervical cord three-dimensional (3D) phase-sensitive inversion recovery (PSIR) MRI scans acquired at baseline and 96 weeks. Spinal cord MRI was part of the optional advanced MRI substudy and was performed at the London site.

- Four measures were derived from DTI acquisition at baseline and at 96 weeks: FA, axial diffusivity (AD), RD and MD. The peak width of skeletonised diffusivity (PWSD) was used as the outcome measure for each metric. This quantifies dispersion of values across all tracts within an individual subject and, therefore, offers improved responsiveness over measures of central tendency in a disease characterised by multifocal pathology. DTI was part of the optional advanced MRI substudy and was performed at the Edinburgh site.

Additional exploratory end points were:

- Measurement of CSF neurofilament levels as part of the CSF substudy at the London site. The primary outcome of the CSF substudy was CSF NfL levels at 48 weeks. A secondary outcome was CSF NfL levels at 96 weeks and the change from 48 to 96 weeks. Additional CSF substudy outcomes included a panel of biomarkers: neural cell adhesion molecule (NCAM), NfH, glial fibrillary acidic protein (GFAP), ferritin, soluble cluster of differentiation 14 (CD14), matrix metalloproteinase 9 (MMP9) and neopterin.
- Measurement of the peripapillary RNFL and retinal nerve GCL and IPL (GCL + IPL complex) with OCT to determine the extent of retinal layer thinning were obtained at baseline and at 96 weeks. OCT scans were acquired as part of the OCT substudy at the London and Edinburgh sites.

Magnetic resonance imaging protocol and analysis

Core magnetic resonance imaging

The following MRI sequences were obtained at all three MRI assessment visits for all participants:

- sagittal localiser to identify the subcallosal line
- axial dual echo fast/turbo spin echo proton density (PD)/T2 weighted from foramen magnum to vertex, in plane resolution 1 mm², contiguous 3-mm slices
- axial fluid attenuated inversion recovery from foramen magnum to vertex, in plane resolution 1 mm², contiguous 3-mm slices
- axial T1 from foramen magnum to vertex, in plane resolution 1 mm², contiguous 3-mm slices
- sagittal 3D T1 gradient echo with voxel resolution of 1 mm³.

Before the beginning of the trial, each site provided a 'dummy scan' (carried out on a healthy volunteer or a person with MS), which was sent to the central MRI facility (Queen Square MS Centre Trial Office, London) for review and agreement on MRI parameters. Detailed MRI parameters according to scanner make and model are reported elsewhere.²⁹ Quality control feedback was generated by review at the central MRI facility soon after scan acquisition and was provided to the site.

The percentage brain volume change was calculated using the SIENA method. After the receipt of digital imaging and communications in medicine images to the central MRI facility and after quality control, the T2 lesions were outlined on PD scans by trained personnel blinded to clinical data using a semi-automatic method (Jim software, version 7.0, Xinapse Systems, Essex, UK). On further MS expert review, T2 lesion masks were used to lesion fill the 3D T1-weighted images using a patch-based method⁸⁶ and, from these, brains were extracted and segmented into GM [i.e. cortical grey matter (CGM) plus deep grey matter (DGM)] and white matter volumes.⁸⁷ Finally, the SIENA method was applied to the 3D T1 images and the segmentations to calculate a percentage change in BV between baseline and week 96 scans.

Percentage change in GM volume was calculated by taking the difference in the GM volumes between 96 weeks and baseline divided by the baseline GM volume.

Advanced magnetic resonance imaging

An advanced MRI protocol was used at the London and Edinburgh sites.

At both the London and Edinburgh sites, the following scans were acquired as part of this advanced protocol:

- brain MTR
- brain MRS.

At the London site only, cervical cord 3D-PSIR scans were performed.

At the Edinburgh site only, brain DTI was performed.

Magnetic resonance spectroscopy

At the Edinburgh site, MRS was carried out at 3 T (Siemens Verio, Siemens Healthcare, Erlangen, Germany) using a standard 12-channel matrix head coil and a manufacturer-supplied prototype with semi-localisation by adiabatic selective refocusing (semi-LASER) excitation. Data were acquired from a single 10-mm-thick slice of spectroscopic voxels prescribed immediately above the lateral ventricles in the plane of the T2-weighted images. Spectroscopic data were analysed in Linear Combination of Model Spectra using a spectral basis set matching the semi-LASER sequence. Voxels were discarded if they had poor-quality spectra, were judged to be not completely inside the brain or the LCModel Cramér–Rao bounds (i.e. % standard deviations) of the fitted spectra exceeded 20% for any metabolite or 40% for myoinositol.⁸⁸

At the London site, the participating patients underwent a standardised MRS sequence on a Philips Achieva 3T MRI scanner (Philips Healthcare, Best, the Netherlands) using a 16-channel neurovascular coil. A 15-mm single-slice-thick volume of interest (VOI) was selected for MRS placed superior to the lateral ventricles. The MRS VOI was subdivided into a 21×16 grid, giving voxel size of $10 \times 10 \times 15$ mm. Spectra were acquired using a 2D Point RESolved Spectroscopy (PRESS) sequence [short echo time (TE) 35 ms/repetition time (TR) 2000 ms]. Outer volume suppression using fat saturation was applied to limit artefacts, and the VOI was shimmed using the pencil beam-auto technique. CHEMical Shift Selective saturation (CHESS) pulses were used for water suppression. A reference scan with no water suppression was also collected with the same parameters in the same examination for quantification. Spectral post-processing analysis was completed using Linear Combination of Model Spectra (LCModel© version 6.3-1A).⁸⁹ Individual voxels were automatically rejected if the NAA, myoinositol and combined glutamate and glutamine (Glx) Cramér–Rao bounds were $> 20\%$, full width half-maximum of the NAA peak was > 1.27 p.p.m. (parts per million) and signal–noise ratio was < 9 . All voxels that passed the automated step were visually inspected by an experienced assessor to look for noisy baseline, non-random residuals or other artefacts that prevented the accurate measurement of neurometabolites.

At both sites, the total concentration of the following metabolites was finally measured: NAA, Glx and myoinositol. The NAA measure included N-acetylaspartylglutamate concentrations.

Magnetisation transfer ratio

Magnetisation transfer ratio data were acquired using a 3D slab-selective fast-field echo sequence with two echo times. Images acquired at the two TEs were averaged (thereby increasing the signal-to-noise ratio) for both the magnetisation transfer on (MTon) and off (MToff) data. The MTR sequences at the two sites (London and Edinburgh) were not identical, in terms of both imaging parameters and MT pulse

characteristics (amplitude, pulse shape, duration, offset frequency). This was unavoidable as they were the sequences acquired on different scanners at the two sites. At the London site, MTR scans were acquired at 3 T using a Philips Achieva scanner with a 32-channel head coil and multitransmit technology. At the Edinburgh site, MTR scans were acquired at 3 T using a Siemens Verio (Siemens Healthcare, Erlangen, Germany) with a 12-channel head coil.

The MTon and MToff images were co-registered to a halfway point using NiftyReg^{90,91} and MTR maps (in percentage units) were calculated as $[(MToff - MTon)/MToff] \times 100$. The T1-weighted volume (in addition to lesion masks, and tissue segmentations for GM and NAWM) were coregistered to this same halfway point for further analysis. Whole-brain, GM, NAWM and T2 lesion masks were measured as part of the core MRI analysis.

Magnetisation transfer ratio maps were reviewed for artefacts, registration and segmentation quality.

Diffusion tensor imaging

Images were acquired at Edinburgh at 3 T (Magnetom Verio, Siemens AG, Healthcare Division GmbH, Erlangen, Germany) using a standard 12-channel head coil. The diffusion imaging protocol consisted of six T2-weighted [T2w ($b = 0 \text{ smm}^{-2}$)] and sets of diffusion-weighted ($b = 1000 \text{ smm}^{-2}$) whole-brain single-shot spin-echo echo-planar imaging volumes acquired with diffusion encoding gradients applied in 56 non-collinear directions. The acquisition parameters were field-of-view $240 \times 240 \text{ mm}$; imaging matrix 96×96 ; 60 contiguous 2.5-mm-thick axial slices, giving 2.5 mm^3 isotropic voxels. Repetition and ETs were 11,500 ms and 73.6 ms, respectively.

Diffusion tensor imaging images were corrected for eddy current-induced distortions and subject motion with the 'eddy correct' tool.⁹² After brain tissue extraction using the Brain Extraction Tool, diffusion tensors and scalar diffusion parameters (FA, MD, AD and RD) were calculated using DTIFit (release 5.0.1. URL: <https://fsl.fmrib.ox.ac.uk/fsl>; accessed 18 June 2018; Analysis Group, FMRIB, Oxford, UK).^{93,94}

Twelve tracts of interest were identified from the diffusion MRI data using probabilistic neighbourhood tractography (PNT) as implemented in the TractoR package for fibre tracking analysis (release 3.1.0. URL: www.tractor-mri.org.uk; accessed 18 June 2018; Jon Clayden, UCL, London, UK). This technique optimises the choice of seed point for tractography by estimating the best matching tract to a reference tract derived from a white matter atlas, using a series of candidate seed points placed in a $7 \times 7 \times 7$ voxel neighbourhood. Tracts assessed were the genu and splenium of the corpus callosum, and bilaterally the cingulum (divided into dorsal and ventral portions), corticospinal tracts, arcuate fasciculi and inferior longitudinal fasciculi. All generated tracts were visually assessed by an experienced observer and those that were deemed not to be anatomically acceptable representations of the fasciculi of interest were discarded from further analysis.

Four metrics were derived from DTI acquisition: FA, AD, RD and MD. The peak width of PWSD was used as the outcome measure for each metric defined as peak width of skeletonised fractional anisotropy (PWSFA), peak width of skeletonised axial diffusivity (PWSAD), peak width of skeletonised radial diffusivity (PWSRD), peak width of skeletonised mean diffusivity (PWSMD). PWSD quantifies dispersion of values across all tracts within an individual subject and, therefore, offers improved responsiveness over measures of central tendency in a disease characterised by multifocal pathology.

Cervical cord magnetic resonance imaging

The MRI of the cervical cord was carried out at the London site only. All the participants were scanned using the same scanner (3 T Philips Achieva system) using a 16-channel neurovascular coil to acquire

spinal cord scans. The following scan was acquired: 3D-PSIR sequence (field of view = 256×256 mm², matrix = 512×256 , TR = 8 ms, TE = 3.7 ms, dual radiofrequency transmit, inversion time = 843.6 ms and number of averaged signals = 3; voxel dimensions $0.5 \times 0.5 \times 3$ mm³ and duration 14 minutes). The cervical cord was imaged in the axial-oblique plane (i.e. slices perpendicular to the cord) containing 16 contiguous slices from C2–C4 with the centre of the imaging volume positioned at the level of C2–C3 intervertebral disc plane.

To calculate the mean upper cord cross-sectional area (MUCCA), an active surface model was used by means of Jim software (version 7.0). To achieve this, we manually marked the centre of five 3-mm-thick slices obtained from the 3D-PSIR image centred at C2–C3, then we ran the Jim software, which identified and contoured the cord edge equivalent to the cord area. Finally, we recorded and averaged the area of each of the five marked slices.⁹⁵ We repeated this procedure for all the baseline and week 96 cervical cord scans to obtain MUCCA for each patient at each single time point. The resulting contouring of the cord cross-sections was visually inspected and manually edited, if necessary.

Optical coherence tomography

Optical coherence tomography imaging was performed on a spectral domain OCT (software version 6.9.4.0 Spectralis, Heidelberg Engineering, Heidelberg, Germany). Room lighting was dimmed and no pharmacological pupil dilation was used. A circular scan [diameter 12°, 1536 A-scans, 1 B-scan, automatic real time (ART) 100] was manually centred on the optic nerve head to acquire a pRNFL image. In addition, to obtain macular images, we acquired volume scans of the macula (London site settings: 20° × 20° volume scan, 25 B-scans, 1024 A-scans per B-scan, vertical alignment, ART 9. Edinburgh site settings: 30° × 25° volume scans, 61 B-scans, 768 A-scans per B-scan, posterior pole alignment, ART 12) centred around the fovea. For the macular scan, we recorded the values using a thickness map on a 1-, 3- and 6-mm grid [Early Treatment Diabetic Retinopathy Study (ETDRS)].⁹⁶ Retinal layer segmentation for quantification of GCL + IPL was obtained by automated segmentation software provided by the manufacturer (Spectralis, Heidelberg Engineering, Heidelberg, Germany).

For the GCL + IPL, we measured both the mean thickness from the ETDRS grid of the inner (i.e. 3-mm ring) and outer (i.e. 6-mm ring) quadrants together and of the inner quadrant alone, the latter being the more reliable measure and, hence, of primary interest.⁹⁷ For the measurement of the pRNFL thickness, the global mean of the entire pRNFL was used, but each single sector was also collected and reviewed for exploratory analyses.

Quality checks were performed in accordance with validated international consensus criteria.⁹⁸ Patients were excluded if they had high refractive error (more than +6 dioptres or less than –6 dioptres) or had concurrent ocular pathology not related to MS. Past history of optic neuritis was recorded. OCTs were performed by trained staff blinded to treatment allocation.

Cerebrospinal fluid

A lumbar puncture for assessment of baseline CSF NfL levels was performed at the baseline visit. A second and third lumbar puncture were then performed at weeks 48 and 96, respectively. Blood and urine samples were collected at baseline, and at weeks 12, 24, 48, 72 and 96. Lumbar punctures were performed according to standard or local practice, using atraumatic needles by trained clinical staff.

Neurofilament light was selected based on the unacceptable interlaboratory coefficient of variation for NfH, compared with the NfL assay⁹⁹ and on the higher propensity of NfH to form aggregates. Finally, we used the commercial SimoaNF-light® assay (Quanterix®, UmanDiagnostics, Ballerica, MA, USA), a digital immunoassay for the quantitative determination of NfL in serum, plasma and CSF.

Sample size

The sample size was calculated based on the study reported by Altmann *et al.*⁴⁹ for measurement of PBVC using the SIENA method in SPMS, and based on further data obtained directly from the lead author (Dr Altmann). Ninety patients per arm would give > 90% power to detect a 40% reduction in PBVC on any active arm compared with placebo and 80% power to detect a 35% reduction, using Bonferroni adjustment for multiple comparisons of three 1.67% two-sided tests, giving 5% overall two-sided significance level. For a more exploratory analysis without adjusting for multiple comparisons, this sample size would give almost 90% power to detect a 35% reduction in atrophy. In addition, based on the experience from two UK Phase II trials,^{100,101} we expected 10% of the total cohort to drop out of the trial before week 48, and a further 10% of the total cohort to come for their week 96 visit, but to be off their medication. According to these figures, a total of 440 patients to be randomised equally (1 : 1 : 1 : 1) between the three active treatments and the placebo (i.e. 110 participants per treatment arm) would anticipate 90 participants per arm to complete the study.

Randomisation and blinding

Sequence generation

Randomisation was by a central, internet-based, secure password-protected randomisation database. The random allocation sequence was generated by the programmers at a UK Clinical Research Collaboration-registered trials unit [Edinburgh Clinical Trials Unit (ECTU)].

Type of randomisation

Patients were randomised to amiloride, fluoxetine, riluzole or placebo in a 1 : 1 : 1 : 1 ratio using a minimisation algorithm balanced according to sex, age (< 45 years or ≥ 45 years), baseline EDSS score (4.0–5.5; 6.0–6.5) and centre, with a random component to maintain unpredictability of treatment allocation.

Implementation

Eligible patients were randomised by study site personnel via a secure web-based randomisation service.

Blinding

Participants and all other personnel directly involved in the study were masked to treatment allocation. Amiloride, fluoxetine, riluzole and placebo capsules were overencapsulated to obtain an identical appearance to ensure that treatment allocation remained concealed to both staff and participants.

The MRI data were processed independently at a central reading site (Queen Square MS Centre Trial Office, UCL, London, UK) by staff unaware of trial group assignments.

Adherence

Adherence was defined as those participants taking, on average, > 90% of prescribed medication as reported in the patient diary in the 30 days prior to their clinic visit, across all clinic visits for which valid data on medication adherence were recorded.

Statistical analysis

Patient recruitment is reported by means of a Consolidated Standards of Reporting Trials (CONSORT) flow diagram. Baseline characteristics of participants are described using exploratory summary methods: continuous variables are summarised using summary statistics (mean, standard deviation, median,

minimum and maximum) by treatment group and categorical variables are presented using frequency distributions by treatment group. Proportions of patients with missing 96-week MRI data in each treatment group are compared, as well as baseline data for patients with missing and non-missing 96-week follow-up data.

Unblinded safety data were monitored by a Data Monitoring Committee (DMC) to ensure the ongoing safety of patients in the study. No formal interim analyses were conducted.

A full statistical analysis plan was prepared that included details of methods for calculating derived variables, methods for handling missing data and withdrawals, any sensitivity analyses and approaches to testing the assumptions in the statistical analyses.

Primary outcome

The primary end point, the PBVC between baseline and 96 weeks, was calculated using a normal linear model to compare the three active treatment group arms with placebo adjusting for baseline normalised BV and the minimisation variables: age, sex, treatment centre (as a fixed effect) and baseline EDSS score. Baseline normalised BV was entered into the model as a continuous variable, as were the minimisation variables of age and EDSS score. Treatment centre was included as an explanatory factor variable with the UCL centre as the reference category. Brighton, Truro and Plymouth each had small numbers of patients (< 11 patients) and so these sites were combined in a 'South Coast Other' category. Similarly, the sites Liverpool, Stoke and Newcastle also had small numbers of patients (< 11 patients) and so were combined into a 'Northern Other' category. The efficacy measure for each active treatment was the mean difference in PBVC change versus placebo. All patients for whom baseline and 96-week BV data were available were included in the analysis according to the treatment group to which they were randomised, irrespective of which treatment(s) they might have received. Dunnett-adjusted 95% simultaneous confidence intervals (CIs) and *p*-values were calculated using a single-step Dunnett procedure in order to adjust for the multiple pairwise comparisons and maintain the overall family-wise error rate of a false-significant result < 5% for the primary outcome analysis. No formal comparison of the active treatments was undertaken.

The primary analysis was a complete-case analysis based on the intention-to-treat (ITT) population. This meant that all patients for whom baseline BV and PBVC at 96-week values were available were included in the analysis according to the treatment group to which they were randomised, regardless of the treatment they actually received. Patients who withdrew from the trial or who were non-compliant with medication were also included, provided baseline BV and PBVC data were recorded for them. In a secondary analysis, the same analysis was based on the per protocol population. The per protocol population included all randomised patients who were adherent to the protocol and compliant with the allocated treatment throughout the duration of follow-up. For this trial, patients were considered compliant with medication if they reported taking, on average, $\geq 90\%$ of their prescribed medication (taking account of planned down-titrations and deferred up-titrations) in the 30 days preceding each clinic visit. Any patients who withdrew from the trial or who were not compliant with medication were excluded from the per protocol population. The per protocol population was formally agreed, prior to database lock, by the chief investigator who was blinded to treatment allocation.

In addition, based on the ITT population defined above, the effect of missing data on the primary outcome analysis was investigated by performing three separate sensitivity analyses.

The sensitivity analyses involved:

1. Missing 96-week PBVC observations were imputed using a pattern-mixture approach to multiple imputation under a missing-not-at-random (MNAR) assumption for missing values.^{102–104} A regression approach was implemented utilising fully conditional specification and the imputation model contained the same variables as for the final analysis. Thirty imputation data sets were created. The imputed

values for PBVC at 96 weeks were then adjusted to reflect the MNAR assumption by adding a constant value equal to the observed standard deviation in the primary outcome at 96 weeks. In the clinical context of MS-SMART, the MNAR concern was that at least some of the missing data at 96 weeks would be due to deteriorating patient outcomes and disability. We report how large an amount can be added to the imputed values without changing the clinical interpretation of the trial, which was suggested as a possible sensitivity analysis for clinical trials by White *et al.*¹⁰⁵ If there were any missing baseline BV values then these will be imputed using the same multiple imputation method but without the MNAR adjustment. In this case we made the missing-at-random (MAR) assumption,¹⁰² which means that given the other data observed in the trial and the statistical analysis model being used, the missingness of baseline BV data are assumed to be independent of baseline BV.

2. A standard multiple imputation analysis was performed assuming that any missing outcome values were MAR. We used the same multiple imputation method as in (1) except no adjustment of imputations with respect to a MNAR assumption was performed in this case.
3. A complete-case approach was used as for the primary analysis except that any outliers more than 4 standard deviations away from the mean were excluded for the PBVC outcome.

Secondary magnetic resonance imaging outcome

Pseudoatrophy

Using the same methods as for the primary analysis, the mean difference in PBVC from baseline to 24 weeks between the placebo group and each of the active treatment groups was also assessed. If the reduction in PBVC was significantly greater in any treatment group, a secondary analysis was performed to compare PBVC from week 24 to week 96 using normal linear modelling. As this was a secondary analysis, no formal adjustment for multiplicity was done.

Counts of new and enlarging T2 lesions

Each active treatment group was compared with placebo in terms of the number of new and enlarging T2 lesions between the baseline and 96-week MRI. Overdispersed Poisson regression models (see Agresti,¹⁰⁶ pp. 149–150) were used to estimate the rate ratio (for each active treatment group compared with placebo) of new/enlarging T2 lesion count after adjusting for baseline T2 lesion volume (T2LV) and the minimisation variables: age, sex, treatment centre and baseline EDSS score.

Secondary clinical outcome

No adjustment for multiplicity was made when analysing the secondary and exploratory end points. The interpretation of secondary and exploratory outcome analyses will be suitably cautious to reflect the high number of outcomes considered.

When the change over time in discrete or continuous outcomes (i.e. EDSS, 9HPT, PASAT, MSFC, SDMT, SLCVA, MSIS29v2, MSWSv2, NFI, NPRS, BPI, NPS and EQ-5D-5L) were found to be reasonably normally distributed, these were compared between the active treatment and placebo groups using normal linear models. As for the primary outcome analysis, the linear models included trial arm as an explanatory factor variable (with placebo as the reference category), the baseline variable (corresponding to the outcome variable) and the minimisation variables: age, gender, treatment centre and baseline EDSS score. Treatment centre was included as an explanatory factor variable with the UCL centre as the reference category. Brighton, Truro and Plymouth each had small numbers of patients (< 11 patients) and so these sites were combined in a 'South Coast Other' category. Similarly, the sites of Liverpool, Stoke and Newcastle also had small numbers of patients (< 11 patients) and so were combined into a 'Northern Other' category. Normality could not be assumed for MSFC and so this outcome was transformed using the signed square root transformation prior to analysis, and results were checked using Mann-Whitney-Wilcoxon non-parametric comparison tests. For the EDSS outcome only, the 95% CIs were computed using a bootstrap method because of the ordinal nature of the outcome variable. Cox proportional hazard models (adjusting for the minimisation variables) were used for time to first relapse and T25FW, with the difference between each active treatment and

placebo being expressed in terms of a hazard ratio. The variable 'time to progress to a given EDSS score' was not analysed using a Cox proportional hazard model as described in the protocol because it was only recorded at a maximum of three time points. Instead, the proportion of trial participants with an increase in EDSS score of at least 1.0 point at 96 weeks relative to baseline was analysed using a multiple logistic regression model adjusting for the minimisation variables.

For the NPS, the same linear regression analysis method was conducted as above, except that the analysis was applied to the individual questionnaire items rather than to any total score because the items are individually meaningful. For question 8 of the NPS, because this question has three categorical options and is in a different format to the other items, frequencies and percentages were calculated for each option at follow-up. Logistic regression was used to determine if there was a significant difference between the active treatment arms and placebo for each of the three binary options separately. These logistic regression models all adjusted for the minimisation variables to enable adjusted odds ratios to be computed.

Corresponding analyses were performed for clinical secondary outcomes measured at 48 weeks.

Exploratory outcomes

Statistical modelling assessed whether baseline MRI or CSF neurofilament levels or disability measures could be used to predict PBVC or EDSS score outcome at 96 weeks. Separate linear regression models were fitted for each potential baseline predictor of PBVC or EDSS score at 96 weeks. Trial arm was included as an explanatory factor variable in each model.

To investigate if treatment effect depends on baseline variables, the same analysis was performed as above, but with an interaction term included in the models representing the interaction between trial arm and the baseline variable. Specifically, we tested for interactions of treatment effect with the following baseline variables: Multiple Sclerosis Walking Scale (MSWSv2), MSIS total (MSIS29v2), MSIS physical, MSIS psychological, cross-sectional area of the upper cervical spinal cord, MSFC, EQ-5D-5L index, T25FW, 9HPT, SDMT score, PASAT, EDSS score at baseline, EDSS score at randomisation, HCVA, SLCVA 5%, SLCVA 2.5%, SLCVA 1.25%, CSF: NfH chain, CSF: NfL chain, T2LV, NAA and N-acetylaspartylglutamate concentration, deep grey matter volume (DGMV), total BV, cortical grey matter volume (CGMV), EQ-5D visual analogue scale (VAS), mean myoinositol concentration, mean Glx concentration, mean T2 hyperintense lesions MTR, mean whole-brain MTR, mean GM MTR, and mean NAWM MTR. Interactions found to be statistically significant suggest that the magnitude of the treatment effect differs according to the value of the baseline variable.

Regarding CSF neurofilament levels, we assessed the unique contribution of neurofilament levels (either NfL or NfH or both together) in predicting PBVC treatment response at 96 weeks over and above other baseline variables that were found to predict treatment response in the above analyses. This consisted of fitting a multiple linear regression model to PBVC at 96 weeks and comparing the model results with and without the neurofilament variables. The R^2 coefficient and residual standard deviation were reported as indicators of model fit.

In addition, a meta-analytic approach was used to assess the value of using MRI or disability or neurofilament variables recorded at 24 or 48 weeks as potential surrogate end points;¹⁰⁷ the rationale being that we aimed to predict the effect of treatment on PBVC/EDSS score at 96 weeks based on the observed treatment effect on a surrogate end point. Bivariate mixed-effects models were used to assess surrogacy separately for each investigational treatment arm versus placebo comparison, based on the estimated treatment effect measured across different centres. Each potential surrogate end point was analysed separately. Coefficients of determination at the centre level were reported for each treatment and each surrogate end point. The analysis was conducted using the package 'Surrogate' within R software [The R Foundation for Statistical Computing, Vienna, Austria; URL: www.r-project.org (accessed 3 February 2020)].

Magnetic resonance imaging

Proportion of new and enlarging T2 lesions at 24 weeks being persistently T1 hypointense at 96 weeks

This outcome was analysed using multiple linear regression adjusting for the minimisation variables.

Percentage grey matter volume change

The percentage of GM volume change was statistically compared between trial arms using a multiple linear regression method adjusting for baseline and the minimisation variables.

Magnetic resonance spectroscopy

Changes in the NAA, myoinositol, and Glx metabolite concentrations were statistically compared between trial arms using a multiple linear regression method adjusting for baseline and the minimisation variables.

Magnetic transfer ratio

Lesion, GM and NAWM MTR measures were statistically compared between trial arms using a multiple linear regression method adjusting for baseline and the minimisation variables.

Diffusion tensor imaging

Peak width of skeletonised diffusivity-FA, AD, RD and MD were statistically compared between trial arms using a multiple linear regression method adjusting for baseline and the minimisation variables.

Cervical cord magnetic resonance imaging

The MUCCA at week 96 was statistically compared between trial arms using a multiple linear regression method adjusting for baseline and the minimisation variables.

Optical coherence tomography

Left and right eye OCT parameters were analysed separately for the pRNFL (primary analysis) and the GCL + IPL. The thickness of the pRNFL was measured from the peripapillary circular OCT scan at baseline and 96 weeks for each eye separately. The primary analysis was the global average pRNFL thickness excluding eyes with optic neuritis. The analysis used a multiple linear regression method adjusting for baseline and the minimisation variables to calculate adjusted mean differences (AMDs) and 95% CIs for the individual pairwise comparisons between each active treatment and placebo. Specific pRNFL sectors of each eye were also analysed using the same approach described above.

Secondary analysis repeated the primary analysis but included all eyes regardless of optic neuritis status, adjusting for the presence of optic neuritis.

The same analyses as above were performed for the macular GCL + IPL thickness volume measured by OCT at 96 weeks.

Other variables from the peripapillary circular scan and the macula volume scan, such as the average macular thickness and volume, were analysed using similar regression methodology.

Cerebrospinal fluid

The primary analysis in the CSF substudy was to determine whether or not there was a reduction in CSF NfL levels at 48 weeks in each of the treatment arms versus placebo arm using multiple linear regression adjusted for baseline and the minimisation variables.

An analysis was also performed for:

- NfL level at 96 weeks adjusting for baseline
- change in NfL from 48 to 96 weeks adjusted for baseline.

Additional analyses (i.e. a panel of biomarkers including NCAM, NfH, GFAP, ferritin, soluble CD14, MMP9 and neopterin) are reported in *Report Supplementary Material 1 (Tables 18 and 19)*.

All analyses listed above were undertaken as complete-case analyses on the ITT population, and were also separately analysed according to the per protocol population based on compliance. All other analyses in this section were undertaken only as complete-case analyses on the ITT population.

Spearman's rank-order correlation coefficients were calculated to assess the bivariate correlation of neurofilament levels with PBVC and EDSS score at 96 weeks.

Safety reporting

All AEs were recorded in the source medical records based on the time a consent form was signed until the end of the study for that participant (this was week 100). Participants were asked whether any AEs or serious adverse events (SAEs) had occurred at each study visit. The data were also extrapolated from written information in the diary and from laboratory results. Symptoms felt to be due to the progression of MS were excluded. Relapses were collated and graded separately.

All SAEs were reported to the sponsor by site investigators within 24 hours of them becoming aware of the event. All suspected unexpected serious adverse reactions (SUSARs) were notified to the sponsor immediately (or at least within 24 hours). The sponsor notified the main REC and Medicines and Healthcare products Regulatory Agency of all SUSARs.

Safety results were reported for the safety analysis population, according to treatment received. The safety analysis population was defined as all patients who were randomised into MS-SMART and who received at least one prescription of study medication. Safety data were analysed according to which treatment was received, rather than the trial arm to which the patient was randomised.

The numbers of AEs, SAEs and SUSARs were reported, split by trial arm. The number and percentage of patients experiencing AEs were also reported. In addition, the AE, SAE and SUSAR tables were presented with the number and percentage of patients recording an event in each of the Medical Dictionary for Regulatory Activities (MedDRA) system organ class categories.

Data monitoring

An independent DMC was established and the terms of reference are reported in a charter that is held in the trial master file (TMF) at ECTU. Unblinded safety data were monitored by the DMC to ensure the ongoing safety of patients in the study. Stopping criteria were not prespecified to the DMC and no formal interim analyses were planned. A Trial Steering Committee (TSC) was established to supervise the conduct and progress of the trial. The terms of reference of the TSC were documented in a charter that was held in the TMF at ECTU.

Summary of protocol changes

There were five protocol amendments that are listed in *Table 1*, resulting in seven versions of the protocol.²⁹

TABLE 1 Protocol amendments

Protocol update	Protocol version	Protocol date	Reason for amendment
NA	V1	1 January 2013	NA
Substantial amendment	V1	1 January 2013	Addition of new sites
Substantial amendment	V2, ^a V3	1 December 2013, 1 June 2014	See note below. Replacement of the drug ibudilast with fluoxetine
Substantial amendment	V4	25 May 2015	Change to eligibility criteria to exclude patients on high-dose simvastatin. Clarification in patient information sheet about side effects of fluoxetine
Substantial amendment	V5	1 November 2016	Protocol updated to reflect changes to fluoxetine summary of product characteristics
Non-substantial amendment	V6	5 October 2017	To update new trials unit address and telephone numbers
Substantial amendment	V6	5 October 2017	To update change of principal investigator
Non-substantial amendment	V7	4 June 2018	To correct typing error of ClinicalTrials.gov number

NA, not applicable.

^a Protocol V2 was submitted for the clinical trial authorisation.

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Chapter 3 Main results

Recruitment

A total of 547 participants were consented and screened between December 2014 and June 2016. The first patient was screened on 18 December 2014 and the first participant was randomised on 29 January 2015. The last patient was randomised on 22 June 2016 and the last patient last visit occurred on 4 July 2018.

Of the 547 patients consented and screened, 445 (81% of total screened) met all eligibility criteria and were consecutively randomised to one of the three active treatments or placebo. A few participants had already undergone the consent process and screening when the target 440th patient had been randomised; therefore, it was appropriate for them to continue in the study and be randomised if eligible. The rates of screening and randomisation are reported in *Figure 4*.

The proportion of patients randomised at each site and the distribution of treatment allocation across sites are reported in *Figure 5*.

Participant flow

A CONSORT flow diagram of recruitment is in *Figure 6*. The 445 eligible participants were randomised to receive amiloride ($n = 111$) or fluoxetine ($n = 111$) or riluzole ($n = 111$) or placebo ($n = 112$).

Two patients randomised to riluzole also received fluoxetine prescribed by their GP during follow-up towards the end of the trial. Only one patient (randomised to riluzole) was in the category of withdrawal by clinician; all other withdrawals were withdrawals by the patient. Two withdrawals (one each from the riluzole and placebo groups) happened shortly after the 96-week MRI scan and so these patients were still included in the primary outcome analysis.

Numbers analysed

Adherence to trial protocol

Only one protocol violation was recorded: eight patients at the Leeds site were randomised prior to baseline data collection. Eligibility was rechecked and all patients were confirmed to be eligible.

Protocol deviations (see *Report Supplementary Material 1, Table 1*) associated with randomised patients were recorded on 317 occasions, with similar frequencies across treatment groups. Most deviations (239; 75%) related to assessments occurring outside protocol-specified time windows. A further six deviations not linked to randomised patients were also recorded.

Adherence to trial medication

Overall, 337 participants were adherent to allocated trial medication. Adherence was similar across treatment groups: amiloride, 83 of 111 (75%); fluoxetine, 87 of 111 (78%); riluzole, 84 of 111 (76%); and placebo, 83 of 112 (74%). Eighty-five participants permanently discontinued their assigned treatment after randomisation [amiloride, 20 (18%); fluoxetine, 24 (22%); riluzole, 22 (20%); and placebo, 19 (17%)].

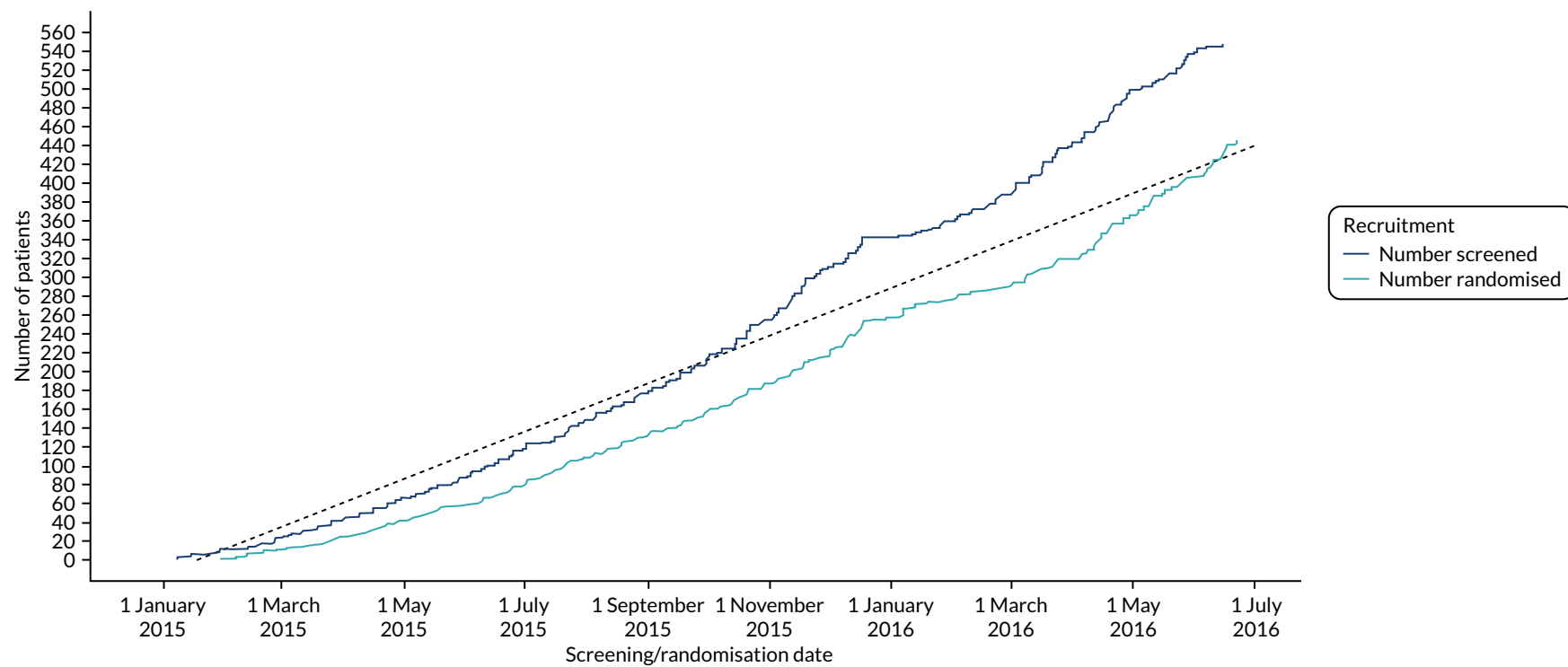


FIGURE 4 Screening and randomisation: cumulative number of patients screened and randomised.

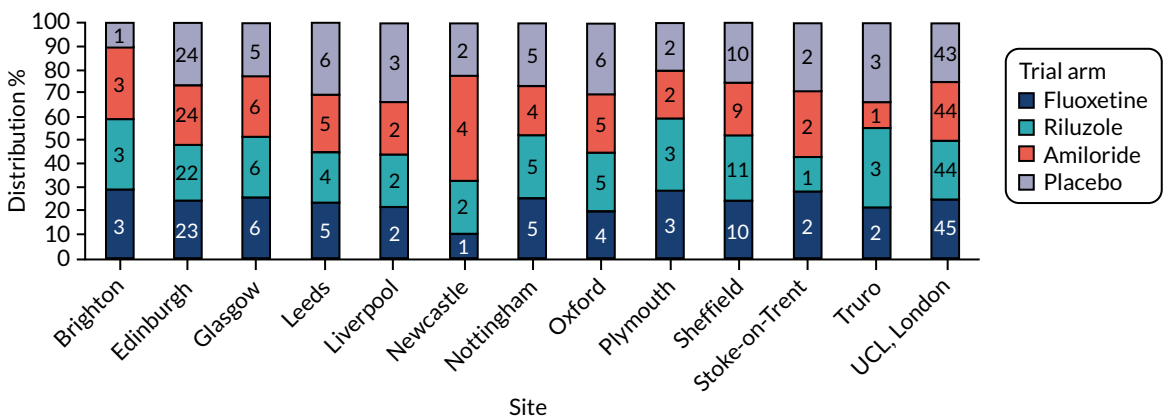


FIGURE 5 Distribution of patients and treatment across the sites.

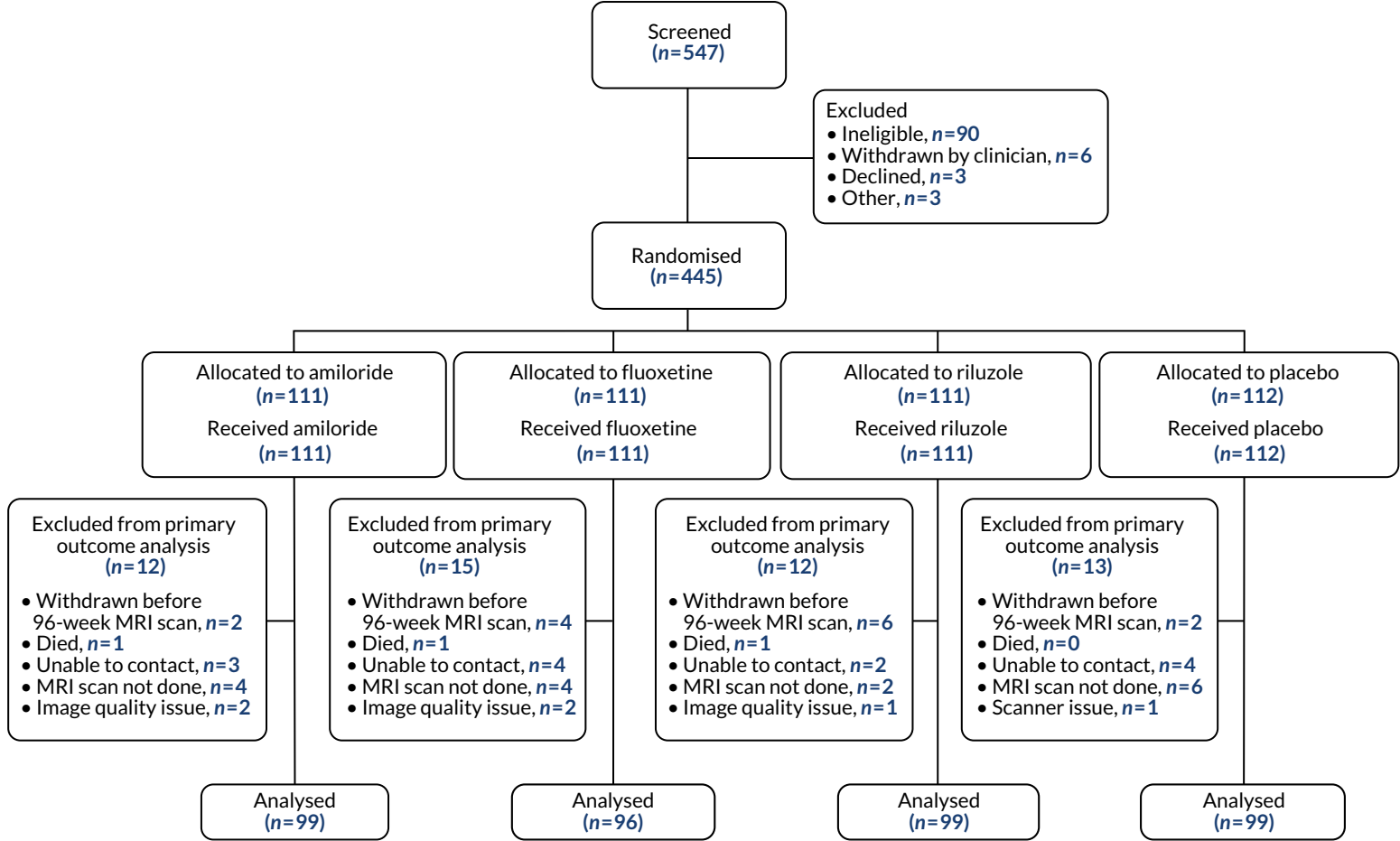


FIGURE 6 Patient disposition. The figure reports the number of participants included in the primary outcome analysis. In total, there were 19 withdrawals from the trial post randomisation. Two withdrawals occurred after the 96-week primary end point and are not reported in this figure.

Adherence to remaining in the trial

Nineteen patients (4%) withdrew from the trial post randomisation (three deceased, one on instruction from their treating clinician and 15 at the request of the participant). Two withdrawals (both at the request of the participant) occurred after the 96-week primary end point. A further 13 patients (3%) could not be contacted and were recorded as lost to follow-up. Fifty-two patients (12%) did not attend the 96-week MRI follow-up: amiloride 12 (11%), riluzole 12 (11%), fluoxetine 15 (14%) and placebo 13 (12%).

Adherence to study blinding

The treatment allocation was unblinded six times during the course of the trial and the reasons were as follows: two deaths (one in the riluzole arm and one in the fluoxetine arm), three SAEs with hospitalisation (two patients were on riluzole, one was on placebo) and one for evidence of clinical worsening, which was suspected to be due to study drugs (the patient was on fluoxetine).

The success or otherwise of the study blinding was assessed via questionnaire for the participant or participant's doctor. A total of 117 out of 386 participant respondents and 202 out of 389 clinician respondents did not venture a guess and selected the 'Don't know' option. Out of those participants making a guess, we found that only 138 out of 269 participants (51%) correctly guessed their treatment allocation. The corresponding kappa statistic was calculated to be 0.039 (95% CI -0.060 to 0.139), which suggests very poor agreement between the participant's guess and the true result. For clinicians, 111 out of 187 (59%) correctly guessed the participant's treatment allocation, corresponding to a kappa statistic of 0.127 (95% CI -0.005 to 0.259). This again suggests poor agreement, albeit the observed agreement was slightly higher for the clinician than for the participant.

Baseline data

Demographic characteristics of the participants are shown in *Table 2*. The clinical characteristics, including EDSS, MSFC with its three subcomponents, SDMT, high and low visual acuity, MSIS29v2 with the two subcomponents and MSWSv2 are reported in *Table 3*.

TABLE 2 Baseline demographic characteristics of participants

	Trial arm				Overall
	Amiloride	Fluoxetine	Riluzole	Placebo	
Age (years)					
n	111	111	111	112	445
Mean	54.36	54.83	54.10	54.89	54.55
SD	7.18	7.10	6.75	7.16	7.03
Gender, n (%)					
Male	36 (32.4)	37 (33.3)	37 (33.3)	37 (33.0)	147 (33.0)
Female	75 (67.6)	74 (66.7)	74 (66.7)	75 (67.0)	298 (67.0)
Race, n (%)					
White	108 (97.3)	105 (94.6)	106 (95.5)	108 (96.4)	427 (96.0)
Black	2 (1.8)	1 (0.9)	1 (0.9)	1 (0.9)	5 (1.1)
Asian	1 (0.9)	4 (3.6)	3 (2.7)	2 (1.8)	10 (2.2)
Other	0 (0)	1 (0.9)	1 (0.9)	1 (0.9)	3 (0.7)
MS disease duration (years)					
n	107	110	109	109	435
Mean	21.40	22.47	21.94	20.73	21.64
SD	9.92	9.59	9.58	9.66	9.68
					continued

TABLE 2 Baseline demographic characteristics of participants (*continued*)

	Trial arm				Overall
	Amiloride	Fluoxetine	Riluzole	Placebo	
SPMS disease duration (years)					
n	111	111	110	111	443
Mean	7.79	7.44	7.86	7.05	7.54
SD	5.51	5.74	5.71	5.66	5.65
Relapse count, number (pre-enrolment)					
n	110	111	111	112	444
Mean	0.19	0.10	0.09	0.15	0.13
SD	0.57	0.36	0.42	0.51	0.47
Median	0	0	0	0	0
Minimum	0	0	0	0	0
Maximum	4	2	3	3	4
SD, standard deviation.					

TABLE 3 Baseline clinical characteristics

		Trial arm				Overall
		Amiloride	Fluoxetine	Riluzole	Placebo	
EDSS, score	<i>n</i>	111	111	111	112	445
	Median	6.0	6.0	6.0	6.0	6.0
	Minimum	4.0	4.0	4.0	4.0	4.0
	Maximum	6.5	6.5	6.5	6.5	6.5
EDSS score band						
4.0–5.5	<i>n</i> (%)	29 (26.1)	28 (25.2)	28 (25.2)	29 (25.9)	114 (25.6)
6.0–6.5	<i>n</i> (%)	82 (73.9)	83 (74.8)	83 (74.8)	83 (74.1)	331 (74.4)
BDI-II	<i>n</i>	111	111	111	112	445
	Median	6	6	7	7	6
	Minimum	0	0	0	0	0
	Maximum	18	18	18	18	18
MSFC, z-score	<i>n</i>	111	111	111	112	445
	Mean	–0.19	–0.02	–0.09	0.00	–0.07
	SD	1.19	0.60	0.95	0.91	0.93
PASAT, number of correct answers	<i>n</i>	111	110	111	112	444
	Mean	39.02	36.60	36.91	41.46	38.51
	SD	13.67	15.17	16.00	13.86	14.78
T25FW, seconds	<i>n</i>	111	109	111	112	443
	Mean	25.14	15.70	19.84	18.63	19.84
	SD	38.84	12.38	28.46	28.53	28.82

TABLE 3 Baseline clinical characteristics (continued)

		Trial arm				Overall
		Amiloride	Fluoxetine	Riluzole	Placebo	
9HPT, sec ⁻¹	<i>n</i>	111	111	111	112	445
	Mean	0.03	0.03	0.03	0.03	0.03
	SD	0.01	0.01	0.01	0.01	0.01
SDMT, number of correct answers	<i>n</i>	109	111	109	112	441
	Median	46	46	45	46.5	46
	Minimum	17	17	18	13	13
	Maximum	72	67	74	75	75
HCVA OD, number of correct answers	<i>n</i>	106	111	110	110	437
	Mean	48.82	52.72	48.78	49.92	50.08
	SD	13.97	7.27	14.84	13.43	12.77
	Median	53.50	53.00	54.00	54.00	54.00
	Minimum	0	25	0	0	0
	Maximum	60	60	60	60	60
HCVA OS, number of correct answers	<i>n</i>	107	111	110	111	439
	Mean	50.09	50.8	48.56	50.42	49.9
	SD	11.21	10.8	14.8	12.71	12.57
	Median	53	54	54	55	54
	Minimum	0	4	0	0	0
	Maximum	62	65	65	64	65
SLCVA 5% OD, number of correct answers	<i>n</i>	106	110	108	109	433
	Mean	31.66	35.15	30.71	34.15	32.94
	SD	14.52	10.46	15.38	14.62	13.93
	Median	35	35	32	38	35
	Minimum	0	0	0	0	0
	Maximum	60	57	56	60	60
SLCVA 5% OS, number of correct answers	<i>n</i>	107	110	108	110	435
	Mean	32.58	32.93	29.96	33.93	32.36
	SD	13.40	12.82	16.13	14.57	14.31
	Median	35.00	34.50	34.50	36.00	35.00
	Minimum	0	0	0	0	0
	Maximum	59	56	55	60	60
SLCVA 2.5% OD, number of correct answers	<i>n</i>	106	111	109	110	436
	Mean	19.90	19.86	19.12	20.74	19.91
	SD	13.15	12.28	14.50	13.69	13.39
	Median	20.50	19.00	18.00	22.00	19.00
	Minimum	0	0	0	0	0
	Maximum	48	50	55	58	58

continued

TABLE 3 Baseline clinical characteristics (continued)

		Trial arm				Overall
		Amiloride	Fluoxetine	Riluzole	Placebo	
SLCVA 2.5% OS, number of correct answers	<i>n</i>	107	111	109	111	438
	Mean	19.10	17.65	18.81	20.85	19.10
	SD	12.71	12.36	14.16	13.98	13.33
	Median	19.00	18.00	20.00	22.00	20.00
	Minimum	0	0	0	0	0
	Maximum	45	39	50	58	58
SLCVA 1.25% OD, number of correct answers	<i>n</i>	106	111	109	110	436
	Mean	9.07	8.94	7.69	9.84	8.88
	SD	10.25	10.19	10.68	11.33	10.62
	Median	5.50	5.00	1.00	5.00	4.00
	Minimum	0	0	0	0	0
	Maximum	42	40	43	42	43
SLCVA 1.25% OS, number of correct answers	<i>n</i>	107	111	109	111	438
	Mean	8.17	6.94	7.08	9.86	8.02
	SD	10.67	9.59	10.66	11.88	10.76
	Median	3.00	2.00	0.00	5.00	2.00
	Minimum	0	0	0	0	0
	Maximum	35	34	43	44	44
MSIS29v2 total score	<i>n</i>	111	111	111	112	445
	Mean	63.91	65	69.15	66.07	66.04
	SD	13.37	13.83	15.01	14.39	14.25
	Median	65.00	64.00	68.00	66.00	65.00
	Minimum	33	32	31	35	31
	Maximum	98	98	106	97	106
MSIS29v2 psychological score	<i>n</i>	111	111	111	112	445
	Mean	15.95	16.66	18.16	17.1	16.97
	SD	4.511	4.76	5.43	4.98	4.98
	Median	15.00	16.00	16.00	17.00	16.00
	Minimum	9	9	9	9	9
	Maximum	32	28	33	30	33
MSIS29v2 physical score	<i>n</i>	111	111	111	112	445
	Mean	47.97	48.34	50.99	48.97	49.07
	SD	10.50	10.44	11.30	11.22	10.90
	Median	48.00	49.00	51.00	48.50	49.00
	Minimum	24	23	21	25	21
	Maximum	70	74	77	74	77

TABLE 3 Baseline clinical characteristics (continued)

		Trial arm				Overall
		Amiloride	Fluoxetine	Riluzole	Placebo	
MSWSv2 total score	<i>n</i>	111	111	110	112	444
	Mean	41.43	41.08	42.64	41.63	41.69
	SD	9.18	9.77	9.32	9.93	9.54
	Median	42.00	44.00	45.00	44.00	43.00
	Minimum	14	14	14	18	14
	Maximum	54	54	54	54	54
EQ-5D-5L index	<i>n</i>	111	111	110	111	443
	Mean	0.68	0.70	0.66	0.67	0.68
	SD	0.17	0.16	0.17	0.18	0.17
	Median	0.73	0.72	0.67	0.72	0.71
EQ-5D-5L VAS: health state score	<i>n</i>	111	111	111	112	445
	Mean	66.13	67.53	61.73	65.24	65.15
	SD	16.90	19.48	21.01	20.33	19.55
	Median	70.00	70.00	65.00	65.00	70.00
NFI summary interval score	<i>n</i>	111	110	109	108	438
	Mean	18.01	17.43	19.05	17.80	18.07
	SD	4.20	3.89	4.80	3.86	4.23
	Median	17.64	17.64	18.45	17.64	17.64
NFI physical interval score	<i>n</i>	111	111	109	110	441
	Mean	15.12	14.66	15.91	14.74	15.10
	SD	3.78	3.87	4.34	3.65	3.93
	Median	14.14	14.14	15.06	14.14	14.14
NFI cognitive interval score	<i>n</i>	111	110	109	109	439
	Mean	6.52	6.21	7.06	6.31	6.52
	SD	2.21	2.24	2.33	2.38	2.31
	Median	6.36	6.36	7.13	6.36	6.36
NFI diurnal interval score	<i>n</i>	111	111	110	110	442
	Mean	10.07	9.61	10.24	9.68	9.90
	SD	2.78	2.93	3.10	2.50	2.84
	Median	10.09	10.09	10.48	10.09	10.09
NFI nocturnal interval score	<i>n</i>	111	111	111	111	444
	Mean	7.75	7.60	8.24	7.96	7.89
	SD	2.04	2.46	2.78	2.29	2.41
	Median	7.83	7.83	8.52	7.83	7.83

OD, right eye; OS, left eye; SD, standard deviation.

MAIN RESULTS

Comorbidities were also recorded and are shown in *Table 4*. Seven conditions of interest were captured: asthma, depression, hypertension, hypothyroidism, hyperlipidaemia, osteoporosis and hysterectomy.

Finally, MRI characteristics (including BV, T2LV, DGMV and CGMV) are reported in *Table 5*.

Overall, the baseline characteristics (including clinical measures, BV and T2LV) were similar across the four trial arms.

TABLE 4 Comorbidities at baseline

Comorbidity	Trial arm, n (%)				Overall, n (%)
	Amiloride	Fluoxetine	Riluzole	Placebo	
Asthma	9 (8.1)	9 (8.1)	5 (4.5)	8 (7.1)	31 (7.0)
Depression	4 (3.6)	6 (5.4)	11 (9.9)	8 (7.1)	29 (6.5)
Hypertension	15 (13.5)	12 (10.8)	17 (15.3)	16 (14.3)	60 (13.5)
Hypothyroidism	7 (6.3)	9 (8.1)	15 (13.5)	10 (8.9)	41 (9.2)
Hyperlipidaemia	11 (9.9)	10 (9)	11 (9.9)	9 (8.0)	41 (9.2)
Osteoporosis	6 (5.4)	7 (6.3)	2 (1.8)	7 (6.3)	22 (4.9)
Hysterectomy	6 (5.4)	5 (4.5)	6 (5.4)	12 (10.7)	29 (6.5)
Other condition	27 (24.3)	25 (22.5)	27 (24.3)	27 (24.1)	106 (23.8)

TABLE 5 Baseline MRI characteristics

		Trial arm				Overall (n = 445)
		Amiloride (n = 111)	Fluoxetine (n = 111)	Riluzole (n = 111)	Placebo (n = 112)	
BV (ml)	Mean	1432.2	1413.1	1414.2	1431.0	1422.6
	SD	84.2	82.4	74.8	91.1	83.6
T2LV (ml)	Mean	13.0	14.1	13.0	13.6	13.4
	SD	12.4	12.7	12.3	12.5	12.4
DGMV (ml)	Mean	45.1	44.0	44.2	45.3	44.7
	SD	4.0	4.1	3.9	4.2	4.1
	Median	45.4	44.0	44.3	44.9	44.7
CGMV (ml)	Mean	794.6	787.5	786.2	791.0	789.8
	SD	40.9	48.3	37.9	49.1	44.3

SD, standard deviation.

Outcomes and estimation

Primary outcome

There was no significant difference between any of the active arms and the placebo arm in the PBVC at week 96 (Table 6 and Figure 7). This finding was confirmed in sensitivity analysis (see Table 6).

The percentage brain volume change at 96 weeks was -1.35% in the overall cohort (see Table 7), corresponding to a PBVC of around -0.7% per year. In the placebo arm, the PBVC was similar to the overall cohort (see Table 7).

TABLE 6 Results from primary outcome analyses

Analysis	n ^a	AMD (%) (active treatment - placebo)	95% CI	Raw p-value	Simultaneous CI	Dunnett- adjusted p-value
Amiloride						
Primary analysis (with Dunnett adjustment)	393	0.036	-0.320 to 0.391	0.843	-0.391 to 0.462	0.995
Sensitivity analysis 1: multiple imputation based on a MNAR assumption ^a	445	0.064	-0.310 to 0.437	0.738	-0.376 to 0.503	0.776
Sensitivity analysis 2: multiple imputation based on a MAR assumption	445	0.039	-0.324 to 0.401	0.834	-0.386 to 0.464	0.858
Sensitivity analysis 3: with outliers removed	392	0.041	-0.305 to 0.387	0.817	-0.374 to 0.456	0.991
Secondary analysis: using per protocol population	263	0.122	-0.278 to 0.523	0.548	-0.360 to 0.605	0.883
Fluoxetine						
Primary analysis (with Dunnett adjustment)	393	-0.116	-0.474 to 0.243	0.527	-0.546 to 0.315	0.863
Sensitivity analysis 1: multiple imputation based on a MNAR assumption ^b	445	-0.137	-0.508 to 0.235	0.471	-0.575 to 0.302	0.541
Sensitivity analysis 2: multiple imputation based on a MAR assumption	445	-0.108	-0.464 to 0.247	0.551	-0.528 to 0.311	0.613
Sensitivity analysis 3: with outliers removed	392	-0.052	-0.402 to 0.298	0.770	-0.472 to 0.368	0.983
Secondary analysis: using per protocol population	263	-0.050	-0.456 to 0.356	0.809	-0.539 to 0.439	0.991
Riluzole						
Primary analysis (with Dunnett adjustment)	393	-0.143	-0.499 to 0.214	0.432	-0.570 to 0.285	0.771
Sensitivity analysis 1: multiple imputation based on a MNAR assumption ^b	445	-0.120	-0.496 to 0.256	0.531	-0.562 to 0.322	0.594

continued

TABLE 6 Results from primary outcome analyses (continued)

Analysis	<i>n</i> ^a	AMD (%) (active treatment – placebo)	95% CI	Raw <i>p</i> -value	Simultaneous CI	Dunnett- adjusted <i>p</i> -value
Sensitivity analysis 2: multiple imputation based on a MAR assumption	445	-0.129	-0.486 to 0.229	0.480	-0.550 to 0.292	0.549
Sensitivity analysis 3: with outliers removed	392	-0.139	-0.486 to 0.207	0.430	-0.555 to 0.277	0.770
Secondary analysis: using per protocol population	263	0.097	-0.312 to 0.505	0.641	-0.395 to 0.588	0.940

a Refers to the overall sample size that the statistical model was based on.

b Conclusions were unchanged regardless of the MNAR assumption. Even when adding or subtracting a very large (unrealistic) value of 20 or 30 to the imputations, statistical significance was absent for all of the treatment effects. Positive AMDs indicate treatment benefit.

The multiple regression model for each outcome included trial arm as an explanatory factor variable (with placebo as the reference category), baseline BV and the minimisation variables: age, gender, treatment centre and EDSS score at randomisation.

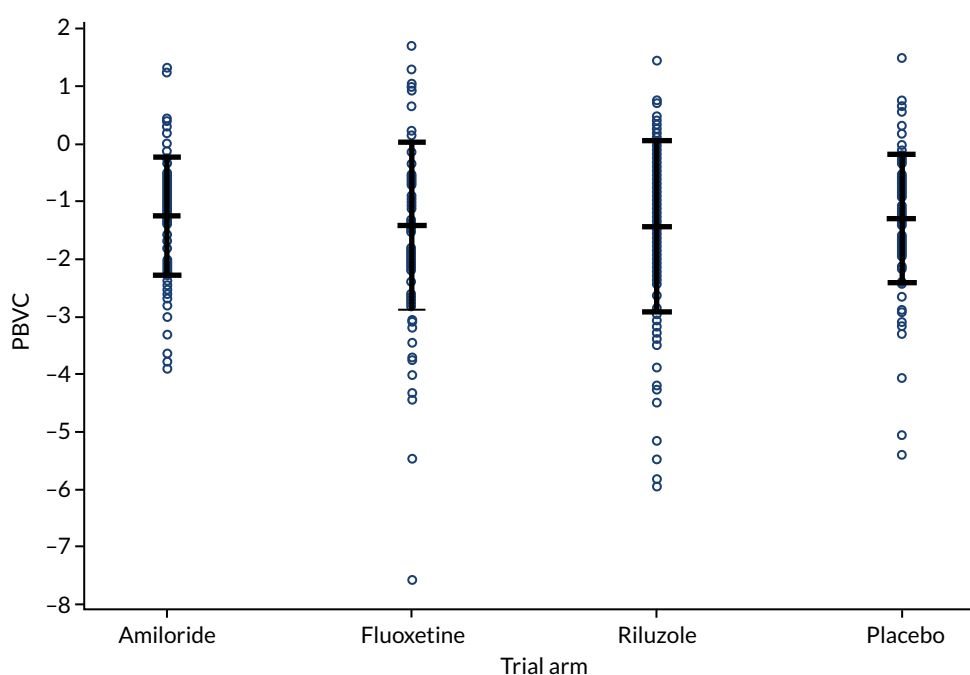


FIGURE 7 Dot plot for PBVC at 96 weeks. Patients are stratified by trial arm. The vertical lines show the mean \pm 1 standard deviation.

Secondary outcomes

Pseudoatrophy

In Table 7, we report the summary of means and standard deviations of PBVC stratified by trial arm.

Percentage brain volume change at 24 weeks was significantly lower in the fluoxetine arm than in the placebo arm (AMD -0.31, 95% CI -0.60 to -0.02; $p = 0.032$), but not for the other active treatment arms versus placebo (see Table 8). There was no difference between any of the active treatment arms and placebo for PBVC between 24 and 96 weeks.

TABLE 7 Summary table of PBVC (%) outcome

PBVC time frame		Trial arm				Overall
		Amiloride	Fluoxetine	Riluzole	Placebo	
PBVC 0–96 weeks	<i>n</i>	99	96	99	99	393
	Mean	–1.25	–1.42	–1.43	–1.29	–1.35
	SD	1.03	1.45	1.48	1.10	1.28
PBVC 0–24 weeks	<i>n</i>	99	104	96	105	404
	Mean	–0.28	–0.59	–0.47	–0.27	–0.40
	SD	0.87	0.90	0.89	0.87	0.89
PBVC 24–96 weeks	<i>n</i>	89	89	89	93	360
	Mean	–0.96	–0.82	–0.86	–1.04	–0.92
	SD	1.02	1.42	1.16	1.17	1.20

SD, standard deviation.

TABLE 8 Percentage brain volume change (%) at 24 weeks and between 24 and 96 weeks

Analysis	<i>n</i>	AMD (%) (active treatment – placebo)	95% CI	Raw <i>p</i> -value	Simultaneous CI	Dunnett-adjusted <i>p</i> -value
Amiloride						
Secondary analysis: PBVC at 24 weeks	404	–0.006	–0.248 to 0.236	0.960	–0.297 to 0.285	1.000
Secondary analysis: PBVC between 24 and 96 weeks	360	0.083	–0.266 to 0.431	0.641	–0.336 to 0.501	0.938
Fluoxetine						
Secondary analysis: PBVC at 24 weeks	404	–0.308	–0.548 to –0.069	0.012	–0.596 to –0.020	0.032
Secondary analysis: PBVC between 24 and 96 weeks	360	0.221	–0.129 to 0.571	0.215	–0.199 to 0.641	0.461
Riluzole						
Secondary analysis: PBVC at 24 weeks	404	–0.179	–0.424 to 0.066	0.151	–0.473 to 0.115	0.342
Secondary analysis: PBVC between 24 and 96 weeks	360	0.150	–0.199 to 0.499	0.397	–0.269 to 0.569	0.733

Bold denotes statistical significance.

The multiple regression model for each outcome included trial arm as an explanatory factor variable (with placebo as the reference category), baseline BV and the minimisation variables: age, gender, treatment centre and EDSS score at baseline. Positive AMDs indicate treatment benefit.

New and enlarging T2 lesions

The number of new and enlarging T2 lesions after 24 and 96 weeks is reported in *Table 9*.

The adjusted rate ratio for the number of new and enlarging T2 lesions detected at the 96-week MRI scan is reported in *Table 10*. An adjusted rate ratio of < 1 indicates a lower rate of new and enlarging T2 lesions in the active treatment arm. The model was based on *n* = 400 patients. The scale parameter of the over-dispersed Poisson model was estimated to be 2.542.

TABLE 9 New and enlarging T2 lesions

New and enlarging T2 lesions		Trial arm				Overall
		Amiloride	Fluoxetine	Riluzole	Placebo	
Number of new and enlarging T2 lesions 0–24 weeks	<i>n</i>	103	106	98	105	412
	Mean	1.11	0.57	0.76	0.85	0.82
	SD	2.61	1.89	1.79	2.84	2.33
Number of new and enlarging T2 lesions 0–96 weeks	<i>n</i>	101	99	100	100	400
	Mean	3.74	1.77	2.83	2.95	2.83
	SD	8.05	5.28	5.71	6.90	6.59

SD, standard deviation.

TABLE 10 Adjusted rate ratio for the number of new and enlarging T2 lesions at 96 weeks

	Adjusted rate ratio for the number of new and enlarging T2 lesions at 96 weeks (active – placebo) and 95% CI ^a	<i>p</i> -value ^a
Amiloride	1.24 (0.83 to 1.83)	0.291
Fluoxetine	0.54 (0.332 to 0.87)	0.012
Riluzole	0.95 (0.63 to 1.45)	0.814

a Adjusted for baseline T2LV and the minimisation variables (age, sex, treatment centre and baseline EDSS score). Bold denotes statistical significance.

There was no significant difference in the number of new and enlarging T2 lesions detected at the 96-week MRI scan for amiloride, or for riluzole versus placebo (Table 10).

Clinical outcomes

Detailed summary statistics for the clinical secondary outcomes at 48 and 96 weeks, split by trial arm, are reported in *Report Supplementary Material 1* (Table 2). Multiple regression analyses for the clinical secondary outcomes at 48 weeks are also reported in *Report Supplementary Material 1* (Table 3).

Clinician-reported outcomes at 96 weeks

Adjusted mean differences for EDSS, MSFC, 9HPT, PASAT, SDMT, SLVCA, T25FW (95% CIs) and *p*-values for each of the treatment arms versus placebo are reported in Table 11.

The multiple regression model for each outcome included trial arm as an explanatory factor variable (with placebo as the reference category), the baseline measurement and the minimisation variables: age, gender, treatment centre and EDSS score at randomisation. Positive AMDs are indicative of observed treatment benefit for all variables except EDSS score.

For the T25FW test, the results of the Cox proportional hazards regression analysis are shown in Table 12. There was no significant difference between any of the treatment arms and placebo.

TABLE 11 Results from secondary clinical outcome analyses

Outcome variable	n	AMD (amiloride – placebo)	95% confidence limits ^a for AMD	p-value	AMD (fluoxetine – placebo)	95% confidence limits ^a for AMD	p-value	AMD (riluzole – placebo)	95% confidence limits ^a AMD	p-value
EDSS, score	383	0.054	–0.124 to 0.223	0.609	–0.067	–0.285 to 0.160	0.535	0.052	–0.158 to 0.245	0.628
9HPT, sec ^{–1}	387	0.000765	–0.000884 to 0.00241	0.362	0.000156	–0.00152 to 0.00183	0.854	0.000933	–0.000742 to 0.00261	0.274
MSFC, ^b z-score	387	0.057	–0.153 to 0.266	0.594	–0.088	–0.299 to 0.124	0.417	0.022	–0.190 to 0.234	0.839
PASAT, number of correct answers	385	0.938	–1.859 to 3.735	0.510	–1.053	–3.898 to 1.792	0.467	0.452	–2.404 to 3.309	0.756
SDMT, number of correct answers	379	–1.030	–3.080 to 1.019	0.324	–1.122	–3.186 to 0.943	0.286	–0.814	–2.899 to 1.270	0.443
HCVA RE, number of correct answers	376	0.901	–1.444 to 3.245	0.450	0.161	–2.190 to 2.513	0.893	–0.116	–2.485 to 2.254	0.924
HCVA LE, number of correct answers	378	3.047	0.486 to 5.609	0.020	1.824	–0.735 to 4.382	0.162	1.631	–0.974 to 4.237	0.219
SLCVA 5% RE, number of correct answers	371	2.007	–0.970 to 4.985	0.186	–0.465	–3.443 to 2.512	0.759	0.351	–2.664 to 3.367	0.819
SLCVA 5% LE, number of correct answers	373	0.999	–1.838 to 3.835	0.489	1.277	–1.568 to 4.122	0.378	1.457	–1.432 to 4.346	0.322
SLCVA 2.5% RE, number of correct answers	375	–0.764	–3.447 to 1.920	0.576	–1.984	–4.663 to 0.695	0.146	–0.542	–3.265 to 2.181	0.696
SLCVA 2.5% LE, number of correct answers	377	0.857	–1.968 to 3.682	0.551	1.306	–1.534 to 4.146	0.366	1.682	–1.191 to 4.556	0.250
SLCVA 1.25% RE, number of correct answers	375	–0.978	–3.126 to 1.170	0.371	–1.925	–4.072 to 0.221	0.079	0.007	–2.177 to 2.190	0.995
SLCVA 1.25% LE, number of correct answers	377	–0.580	–2.658 to 1.498	0.583	–0.797	–2.892 to 1.298	0.455	0.742	–1.373 to 2.856	0.491

LE, left eye; RE, right eye.

^a CIs calculated using 1000 bootstrap resamples for EDSS score outcome.^b MSFC was signed-square root transformed prior to analysis. The linear model for each outcome included trial arm as an explanatory factor variable (with placebo as the reference category), the baseline measurement and the minimisation variables: age, gender, treatment centre and EDSS score at randomisation.

Bold denotes statistical significance.

TABLE 12 Results from the T25FW test analysis at 96 weeks

<i>n</i> = 382	Adjusted hazard ratio	95% CI	<i>p</i> -value
Amiloride vs. placebo	0.824	0.608 to 1.117	0.213
Fluoxetine vs. placebo	0.807	0.595 to 1.096	0.170
Riluzole vs. placebo	0.835	0.615 to 1.135	0.249
Adjusted for the minimisation variables. Hazard ratio > 1 indicates treatment benefit.			

Patient-reported outcomes

Adjusted mean differences for MSIS29v2, MSWSv2, EQ-5D-5L and NFI (95% CIs) and *p*-values for each of the treatment arms versus placebo are reported in *Table 13*. AMDs for pain questionnaires (i.e. BPI, NPRS, NPS) are reported in *Report Supplementary Material 1 (Table 4)*.

The multiple regression model for each outcome included trial arm as an explanatory factor variable (with placebo as the reference category), the baseline measurement and the minimisation variables: age, gender, treatment centre and EDSS score at randomisation. Negative AMDs are indicative of observed treatment benefit for all variables except EQ-5D.

One-point increase in EDSS score at 96 weeks

For the calculation of the 1-point EDSS score increase, the fitted logistic regression model was unstable and had problems with convergence and so centre was recategorised into four categories when fitting the model: London, Edinburgh, Sheffield and Other, with 'London' as the reference category. Results from this analysis are shown in *Table 14*. There was no significant difference between the three treatment arms and placebo.

Relapses

Fifty-one patients (11%) experienced at least one relapse overall during follow-up. For each trial arm, patients experienced at least one relapse as follows: amiloride, *n* = 16 (14%); fluoxetine, *n* = 10 (9%); riluzole, *n* = 11 (10%); and placebo, *n* = 14 (12%).

Analysis from the Cox proportional hazards regression model showed no significant difference in the time to first relapse for any of the active arms compared with placebo (*Table 15*).

The corresponding results for the clinical outcomes at 48 weeks are shown in *Report Supplementary Material 1 (Table 3)*.

Safety

There were no emergent safety issues in the four trial arms. *Table 16* shows the number of patients experiencing AEs, split by treatment received and MedDRA category.

Table 17 shows the number of SAEs, split by treatment received and MedDRA category.

TABLE 13 Results from most relevant patient-reported outcome analyses at 96 weeks

Outcome variable	n	AMD (amiloride – placebo)	95% confidence limits for AMD	p-value	AMD (fluoxetine – placebo)	95% confidence limits for AMD	p-value	AMD (riluzole – placebo)	95% confidence limits for AMD	p-value
MSIS29v2 – total score	390	3.697	0.233 to 7.161	0.037	0.468	–3.035 to 3.972	0.793	0.941	–2.582 to 4.464	0.600
MSIS29v2 – physical score	392	2.187	–0.333 to 4.707	0.089	0.745	–1.799 to 3.289	0.565	0.573	–1.991 to 3.136	0.661
MSIS29v2 – psychological score	390	1.505	0.193 to 2.816	0.025	–0.276	–1.600 to 1.048	0.682	0.463	–0.865 to 1.791	0.494
MSWSv2 score	390	0.466	–1.608 to 2.540	0.659	0.997	–1.112 to 3.106	0.353	0.645	–1.467 to 2.757	0.549
EQ-5D index score	390	–0.010	–0.057 to 0.038	0.690	–0.016	–0.064 to 0.033	0.524	–0.006	–0.054 to 0.043	0.820
EQ-5D VAS score	391	–2.052	–7.587 to 3.483	0.466	–3.002	–8.617 to 2.613	0.294	–2.991	–8.635 to 2.653	0.298
NFI summary interval score	385	0.912	–0.204 to 2.029	0.109	0.527	–0.601 to 1.655	0.359	0.748	–0.393 to 1.888	0.198
NFI physical interval score	388	1.241	0.204 to 2.278	0.019	0.727	–0.321 to 1.776	0.173	1.013	–0.048 to 2.075	0.061
NFI cognitive score	387	0.100	–0.404 to 0.604	0.696	0.155	–0.354 to 0.664	0.549	0.157	–0.359 to 0.674	0.549
NFI diurnal interval score	384	–0.288	–0.904 to 0.329	0.359	–0.336	–0.954 to 0.283	0.286	–0.270	–0.893 to 0.353	0.395
NFI nocturnal interval score	390	0.636	0.134 to 1.138	0.013	0.398	–0.109 to 0.905	0.124	0.116	–0.392 to 0.624	0.653

Bold denotes statistical significance.

TABLE 14 Expanded Disability Status Scale score increase

<i>n</i> = 383	Adjusted odds ratio	95% CI	<i>p</i> -value
Amiloride vs. placebo	0.845	0.349 to 2.029	0.705
Fluoxetine vs. placebo	0.999	0.422 to 2.369	0.999
Riluzole vs. placebo	1.168	0.504 to 2.729	0.717
Adjusted for the minimisation variables. Adjusted odds ratios < 1 indicate treatment benefit.			

TABLE 15 Time to first relapse

<i>n</i> = 445	Adjusted hazard ratio	95% CI	<i>p</i> -value
Amiloride vs. placebo	1.145	0.557 to 2.354	0.713
Fluoxetine vs. placebo	0.737	0.327 to 1.661	0.461
Riluzole vs. placebo	0.784	0.355 to 1.731	0.547
Adjusted for the minimisation variables. Adjusted hazard ratios < 1 indicate treatment benefit.			

TABLE 16 Adverse events

MedDRA category	Treatment received					Total
	Amiloride	Fluoxetine	Riluzole	Riluzole + fluoxetine ^a	Placebo	
Total number of patients	111	111	109	2	112	445
Blood and lymphatic system disorders, <i>n</i> (%)	5 (5)	3 (3)	2 (2)	0	3 (3)	13 (3)
Cardiac disorders, <i>n</i> (%)	1 (1)	3 (3)	8 (7)	0	2 (2)	14 (3)
Ear and labyrinth disorders, <i>n</i> (%)	5 (5)	3 (3)	1 (1)	0	5 (4)	14 (3)
Endocrine disorders, <i>n</i> (%)	0	0	1 (1)	0	0	1 (< 1)
Eye disorders, <i>n</i> (%)	13 (12)	8 (7)	9 (8)	0	8 (7)	38 (9)
Gastrointestinal disorders, <i>n</i> (%)	46 (41)	62 (56)	49 (45)	0	36 (32)	193 (43)
General disorders and administration, <i>n</i> (%)	26 (23)	28 (25)	27 (25)	1 (50)	32 (29)	114 (26)
Hepatobiliary disorders, <i>n</i> (%)	2 (2)	3 (3)	0	0	1 (1)	6 (1)
Immune system disorders, <i>n</i> (%)	1 (1)	1 (1)	3 (3)	0	0	5 (1)
Infections and infestations, <i>n</i> (%)	68 (61)	58 (52)	62 (57)	0	69 (62)	257 (58)
Injury, poisoning and procedural complications, <i>n</i> (%)	26 (23)	43 (39)	29 (27)	1 (50)	28 (25)	127 (29)
Investigations, <i>n</i> (%)	10 (9)	20 (18)	17 (16)	0	8 (7)	55 (12)
Metabolism and nutrition disorders, <i>n</i> (%)	2 (2)	9 (8)	7 (6)	0	4 (4)	22 (5)
Musculoskeletal and connective tissue disorders, <i>n</i> (%)	37 (33)	26 (23)	37 (34)	1 (50)	29 (26)	130 (29)
Neoplasms benign, malignant and unspecified, <i>n</i> (%)	2 (2)	1 (1)	4 (4)	0	2 (2)	9 (2)
Nervous system disorders, <i>n</i> (%)	48 (43)	46 (41)	47 (43)	0	44 (39)	185 (42)
Psychiatric disorders, <i>n</i> (%)	21 (19)	30 (27)	22 (20)	0	22 (20)	95 (21)
Renal and urinary disorders, <i>n</i> (%)	9 (8)	13 (12)	10 (9)	0	5 (4)	37 (8)

TABLE 16 Adverse events (continued)

MedDRA category	Treatment received					Total
	Amiloride	Fluoxetine	Riluzole	Riluzole + fluoxetine ^a	Placebo	
Reproductive system and breast disorders, <i>n</i> (%)	4 (4)	3 (3)	2 (2)	0	2 (2)	11 (2)
Respiratory disorders, <i>n</i> (%)	15 (14)	23 (21)	13 (12)	0	16 (14)	67 (15)
Skin and subcutaneous tissue disorders, <i>n</i> (%)	16 (14)	11 (10)	13 (12)	2 (100)	17 (15)	59 (13)
Surgical and medical procedures, <i>n</i> (%)	6 (5)	3 (3)	8 (7)	0	7 (6)	24 (5)
Vascular disorders, <i>n</i> (%)	4 (4)	2 (2)	3 (3)	0	6 (5)	15 (3)
^a This column refers to two patients randomised to riluzole who also received fluoxetine prescribed by their GP during follow-up.						

TABLE 17 Serious adverse events

MedDRA category	Trial arm (<i>n</i>)				Total (<i>n</i>)
	Amiloride	Fluoxetine	Riluzole	Placebo	
Cardiac disorders	0	1	3	0	4
Gastrointestinal disorders	2	0	0	1	3
General disorders and administration	0	2	1	1	4
Hepatobiliary disorders	2	1	0	3	6
Infections and infestations	5	1	4	4	14
Injury, poisoning and procedural complications	5	0	3	2	10
Investigations	0	0	0	2	2
Musculoskeletal and connective tissue disorders	0	1	0	0	1
Neoplasms benign, malignant and unspecified	1	0	0	0	1
Nervous system disorders	1	0	1	0	2
Psychiatric disorders	0	1	1	1	3
Renal and urinary disorders	1	1	0	0	2
Respiratory disorders	1	0	1	0	2
Skin and subcutaneous tissue disorders	0	0	0	1	1
Surgical and medical procedures	1	0	1	1	3
Vascular disorders	0	0	0	1	1
Total	19	8	15	17	59

There was only one SUSAR; this was a hospitalisation due to suspected drug toxicity and the MedDRA category was 'Injury, poisoning and procedural complications'. The patient was randomised to the riluzole treatment arm.

Three deaths occurred unrelated to the investigational agents; for two patients, death was unexpected and the cause of death remained unknown and one patient died of metastatic lung cancer.

Chapter 4 Substudy and exploratory outcome results

There were 244 patients originally consented to the advanced MRI substudy, 308 to the OCT substudy and 84 to the CSF substudy. There were 206 patients randomised to the advanced MRI substudy, 260 to the OCT substudy and 70 to the CSF substudy.

Magnetic resonance imaging

Summary statistics for the exploratory and advanced MRI substudy outcomes at baseline and 96 weeks are reported in *Report Supplementary Material 1, Table 6*. AMDs in the 96-week measures between each active treatment arm and placebo are shown in *Table 18*.

The MRI outcome of 'Proportion of new and enlarging T2 lesions at 24 weeks being persistently T1 hypointense at 96 weeks' was calculated as 'Count of T1 hypointense lesions at week 96 which have a corresponding new or enlarging T2 hyperintense lesion at week 24' divided by 'Count of new and enlarging T2 hyperintense lesions since baseline at 24 weeks'. There was no significant difference in the proportion of new and enlarging T2 lesions at 24 weeks being persistently T1 hypointense at 96 weeks between the three active arms and placebo (*Table 19*).

Optical coherence tomography

Summary statistics for the OCT left eye and right eye, pRNFL and GCL + IPL outcomes for all patients' eyes and for patients' eyes without previous history of optic neuritis at baseline and at 96 weeks are reported in *Report Supplementary Material 1, Tables 7–14*.

For pRNFL, left eye measures were obtained from 159 participants (119 participants had not suffered from previous optic neuritis) and right eye measures from 163 participants (113 participants had not suffered from previous optic neuritis).

For GCL + IPL, left eye measures were obtained from 161 participants (121 participants had not suffered from previous optic neuritis); and right eye measures from 165 participants (117 participants had not suffered from previous optic neuritis).

The AMDs between active drug and placebo for pRNFL thickness and complex GCL + IPL thickness are shown in *Table 20*.

Secondary analyses including individual pRNFL sector thickness, as well as analyses of the global (inner plus outer) macular thickness from the ETDRS grid, are shown in *Report Supplementary Material 1, Table 15*.

Cerebrospinal fluid

Summary statistics of the CSF substudy at week 48 and week 96 are reported in the supplementary material (see *Report Supplementary Material 1, Tables 16 and 17*). The results of the panel of additional biomarkers (i.e. NCAM, NfH, GFAP, ferritin, soluble CD14, MMP9 and neopterin) are also described in the supplementary material (see *Report Supplementary Material 1, Tables 18–20*).

TABLE 18 Exploratory and advanced MRI outcomes

Outcome variable	n	AMD (amiloride - placebo)	95% confidence limits for AMD	p-value	AMD (fluoxetine - placebo)	95% confidence limits for AMD	p-value	AMD (riluzole - placebo)	95% confidence limits for AMD	p-value
Percentage DGM volume change (%)	380	-0.371	-0.815 to 0.074	0.102	0.085	-0.364 to 0.535	0.709	-0.304	-0.752 to 0.144	0.183
Percentage CGM volume change (%)	380	-0.067	-0.430 to 0.295	0.716	-0.420	-0.786 to -0.054	0.025	-0.187	-0.551 to 0.178	0.314
Mean NAA concentration (London) (millimolar units)	121	0.060	-0.278 to 0.398	0.725	0.124	-0.233 to 0.481	0.492	-0.180	-0.521 to 0.161	0.297
Mean NAA concentration (Edinburgh) (millimolar units)	36	219.980	14.452 to 425.507	0.037	110.551	-70.491 to 291.593	0.221	110.623	-86.264 to 307.510	0.259
Myoinositol concentration (London) (millimolar units)	121	-0.082	-0.305 to 0.140	0.464	-0.252	-0.485 to -0.019	0.034	0.004	-0.220 to 0.229	0.969
Myoinositol concentration (Edinburgh) (millimolar units)	36	-6.058	-78.309 to 66.194	0.865	18.642	-43.733 to 81.018	0.545	0.606	-67.951 to 69.163	0.986
Glx concentration (London) (millimolar units)	121	-0.165	-0.613 to 0.282	0.465	-0.099	-0.568 to 0.370	0.676	-0.195	-0.648 to 0.259	0.397
Glx concentration (Edinburgh) (millimolar units)	36	38.947	-119.41 to 197.304	0.618	2.508	-138.545 to 143.561	0.971	39.327	-112.318 to 190.972	0.599
Whole-brain MTR (mean)	112	-0.065	-1.212 to 1.082	0.911	-0.008	-1.237 to 1.221	0.990	0.191	-0.944 to 1.327	0.739
GM MTR (mean)	112	-0.175	-1.375 to 1.026	0.774	0.156	-1.131 to 1.442	0.811	0.086	-1.104 to 1.276	0.887
NAWM MTR (mean)	112	-0.237	-1.375 to 0.901	0.680	0.171	-1.049 to 1.391	0.782	0.266	-0.861 to 1.392	0.641
T2L MTR (mean)	112	-0.386	-1.960 to 1.187	0.627	0.197	-1.493 to 1.887	0.818	0.912	-0.647 to 2.470	0.249
MUCCA (mm ²)	122	-0.445	-1.303 to 0.414	0.307	-0.372	-1.262 to 0.518	0.410	-0.587	-1.458 to 0.284	0.185
PWSAD (mm ² /second)	35	-0.204	-0.682 to 0.273	0.388	-0.304	-0.681 to 0.073	0.109	-0.009	-0.420 to 0.401	0.963
PWSFA (scaled between 0 and 1)	35	-0.00999	-0.0283 to 0.00829	0.272	-0.00845	-0.0226 to 0.00573	0.232	0.000349	-0.0149 to 0.0156	0.963
PWSMD (mm ² /second)	35	-0.145	-0.464 to 0.173	0.358	-0.199	-0.454 to 0.057	0.122	0.178	-0.102 to 0.457	0.204
PWSRD (mm ² /second)	35	-0.220	-0.462 to 0.022	0.073	-0.233	-0.431 to -0.035	0.023	0.095	-0.121 to 0.311	0.376

T2L, T2 lesion.

Spectroscopy metabolites were measured with water reference scaling. Metabolite concentrations are reported in millimolar units. Positive AMDs for percentage DGM and CGM volume change indicate treatment benefit.

TABLE 19 Count of T1 hypointense lesions at week 96

Treatment effect	AMD (active treatment – placebo)	95% confidence limits for AMD	p-value
Amiloride	0.072	–0.160 to 0.304	0.538
Fluoxetine	0.065	–0.191 to 0.321	0.616
Riluzole	–0.004	–0.250 to 0.242	0.974
Negative AMDs indicate treatment benefit.			

A total of 39 participants (fluoxetine, $n = 11$; riluzole, $n = 9$; amiloride, $n = 10$; placebo, $n = 9$) completed the CSF add-on study. The baseline NfL and NfH values were on average similar across the groups: NfL mean range 829–1079 pg/ml and NfH mean range 452–479 pg/ml (Table 21).

Predictive modelling of the primary and Expanded Disability Status Scale outcomes according to baseline magnetic resonance imaging/disability scores

As part of the exploratory analyses, additional statistical modelling scores explored whether or not baseline MRI/disability could be used to predict temporal evolution of SPMS.

Separate linear regression models were fitted for each potential baseline predictor of PBVC at 96 weeks and EDSS score at 96 weeks and the results are shown in Tables 22 and 23, respectively. Trial arm was included as an explanatory factor variable in each model. Tables 22 and 23 are ordered according to the magnitude of the standardised coefficient (largest to smallest).

A high number of variables were statistically significant and/or had a high standardised coefficient as shown in Tables 22 and 23. In further analysis, we investigated the effect of multiple baseline variables at once on PBVC at 96 weeks.

In model 1 we included (1) all baseline variables that were statistically significant with $p < 0.05$, (2) any variables with standardised coefficient greater than the lowest standardised coefficient among the baseline variables with $p < 0.05$ (PASAT) and (3) MSFC, which appeared predictive of outcome after including interaction terms. The model, therefore, included 14 variables: SLCVA 2.5% right eyes, SLCVA 5% right eyes, SDMT, mean myoinositol concentration (London), HCVA right eyes, SLCVA 2.5% left eyes, SLCVA 1.25% right eyes, HCVA left eyes, mean T2 lesion MTR, SLCVA 5% left eyes, SLCVA 1.25% left eyes, T2LV, PASAT and MSFC. PBVC at 96 weeks was the outcome variable. The fitted model ($n = 117$) produced an R^2 value of 0.220, adjusted R^2 value of 0.086 and residual standard deviation of 1.416.

The advanced MRI variables were found to have a substantially lower sample size than other variables, and so in order to maximise the available sample size we repeated the analysis after removing the advanced MRI variables with a sample size below 380. To further simplify the model, we also excluded SLCVA 5% right eyes, SLCVA 5% left eyes and SLCVA 1.25% right eyes because they were strongly correlated with at least one other variable with a higher standardised coefficient (correlation greater than 0.75). Therefore, in model 2 we included nine variables in total: SLCVA 2.5% right eyes, SDMT, HCVA right eyes, SLCVA 2.5% left eyes, HCVA left eyes, SLCVA 1.25% left eyes, T2LV, PASAT and MSFC. Again, PBVC at 96 weeks was the outcome variable. This model ($n = 383$) produced an R^2 value of 0.060, adjusted R^2 value of 0.029 and residual standard deviation of 1.254.

TABLE 20 Optical coherence tomography results

Outcome variable	n	AMD (amiloride – placebo)	95% confidence limits for AMD	p-value	AMD (fluoxetine – placebo)	95% confidence limits for AMD	p-value	AMD (riluzole – placebo)	95% confidence limits for AMD	p-value
<i>Eyes with no optic neuritis only</i>										
pRNFL thickness (µm) LE (primary OCT analysis)	119	-0.819	-2.245 to 0.607	0.258	0.210	-1.126 to 1.547	0.756	-0.982	-2.387 to 0.422	0.169
pRNFL thickness (µm) RE (primary OCT analysis)	113	-0.260	-1.334 to 0.814	0.632	0.961	-0.072 to 1.993	0.068	-0.868	-1.922 to 0.187	0.106
GCL + IPL thickness (µm) LE	121	0.028	-0.948 to 1.004	0.955	0.202	-0.723 to 1.127	0.666	-0.100	-1.066 to 0.865	0.837
GCL + IPL thickness (µm) RE	117	-0.071	-0.905 to 0.764	0.867	0.375	-0.466 to 1.216	0.379	0.120	-0.763 to 1.004	0.788
<i>All eyes, adjusted for optic neuritis</i>										
pRNFL thickness (µm) LE	159	-0.870	-2.080 to 0.339	0.157	0.429	-0.731 to 1.589	0.466	-1.053	-2.240 to 0.133	0.082
pRNFL thickness (µm) RE	163	-0.037	-0.901 to 0.827	0.933	1.033	0.236 to 1.829	0.011	-0.408	-1.221 to 0.404	0.323
GCL + IPL thickness (µm) LE	161	0.248	-0.630 to 1.127	0.577	0.235	-0.614 to 1.084	0.585	0.097	-0.762 to 0.956	0.824
GCL + IPL thickness (µm) RE	165	0.568	-0.340 to 1.475	0.218	1.015	0.117 to 1.913	0.027	0.829	-0.078 to 1.736	0.073
LE, left eye; RE, right eye. Only the inner sectors of the ETDRS grid contributed to the calculation of the GCL + IPL thickness. Positive AMDs indicate treatment benefit.										

TABLE 21 Cerebrospinal fluid results

Outcome variable	n	AMD (amiloride – placebo)	95% confidence limits for AMD	p-value	AMD (fluoxetine – placebo)	95% confidence limits for AMD	p-value	AMD (riluzole – placebo)	95% confidence limits for AMD	p-value
NfL at 48 weeks, pg/ml	39	75.22	–173.72 to 324.15	0.542	149.30	–92.75 to 391.36	0.218	93.21	–162.06 to 348.47	0.462
NfH at 48 weeks, pg/ml	39	–20.34	–91.10 to 50.42	0.562	52.22	–16.82 to 121.26	0.133	41.74	–30.57 to 114.04	0.248
NfL at 96 weeks, pg/ml	35	–415.85	–1118.12 to 286.42	0.235	–186.00	–897.99 to 525.99	0.596	–371.05	–1113.65 to 371.55	0.314
NfH at 96 weeks, pg/ml	35	–232.83	–558.09 to 92.43	0.153	–190.60	–523.54 to 142.35	0.250	–205.35	–562.45 to 151.75	0.248
NfL change from 48 to 96 weeks, pg/ml	32	–685.92	–1305.60 to –66.24	0.031	–515.86	–1136.49 to 104.78	0.099	–538.27	–1200.42 to 123.88	0.106
NfH change from 48 to 96 weeks, pg/ml	32	–296.13	–639.75 to 47.49	0.088	–314.46	–661.71 to 32.80	0.074	–334.01	–711.92 to 43.90	0.081
PPP: NfL at 48 weeks, pg/ml	23	71.45	–334.35 to 477.25	0.713	241.70	–235.29 to 718.69	0.297	68.90	–335.24 to 473.05	0.721
PPP: NfH at 48 weeks, pg/ml	23	–15.49	–106.08 to 75.10	0.721	59.54	–45.90 to 164.99	0.247	63.45	–27.80 to 154.69	0.159
PPP: NfL at 96 weeks, pg/ml	22	168.47	–275.83 to 612.77	0.430	469.27	–39.78 to 978.32	0.068	339.48	–120.71 to 799.66	0.136
PPP: NfH at 96 weeks, pg/ml	22	101.73	–23.61 to 227.06	0.104	149.06	3.23 to 294.89	0.046	88.48	–44.47 to 221.42	0.175
PPP: NfL change from 48 to 96 weeks, pg/ml	21	–92.63	–517.51 to 332.25	0.645	66.22	–415.68 to 548.13	0.77	–60.23	–481.22 to 360.76	0.762
PPP: NfH change from 48 to 96 weeks, pg/ml	21	123.95	20.19 to 227.72	0.023	96.15	–20.71 to 213.00	0.10	4.12	–102.70 to 110.94	0.935
PPP, per protocol population. Negative AMDs indicate treatment benefit.										

TABLE 22 Investigation of baseline predictors of PBVC at 96 weeks

Baseline variable	n	Coefficient	95% CI	p-value	Standardised coefficient	R ²
NfH (pg/ml)	49	-0.0033	-0.0057 to -0.0009	0.0076	-0.382	0.184
NfL (pg/ml)	49	-0.0009	-0.0017 to -0.0001	0.0200	-0.347	0.151
SLCVA 2.5% RE (number of correct answers)	385	0.0191	0.0095 to 0.0288	0.0001	0.196	0.043
SLCVA 5% RE (number of correct answers)	382	0.0166	0.0072 to 0.0259	0.0006	0.177	0.036
SDMT (number of correct answers)	389	0.0170	0.0069 to 0.0271	0.0010	0.167	0.031
Mean myoinositol (London) (millimolar units)	133	-0.3714	-0.7560 to 0.0132	0.0583	-0.164	0.066
HCVA RE (number of correct answers)	386	0.0163	0.0059 to 0.0266	0.0022	0.157	0.028
SLCVA 2.5% LE (number of correct answers)	387	0.0150	0.0054 to 0.0247	0.0024	0.155	0.028
SLCVA 1.25% RE ^a (number of correct answers)	385	0.0181	0.0061 to 0.0301	0.0032	0.151	0.027
HCVA LE (number of correct answers)	388	0.0150	0.0048 to 0.0252	0.0040	0.147	0.025
T2L MTR (mean)	156	0.0307	-0.0034 to 0.0649	0.0772	0.142	0.040
SLCVA 5% LE (number of correct answers)	384	0.0124	0.0033 to 0.0215	0.0074	0.137	0.023
SLCVA 1.25% LE (number of correct answers)	387	0.0160	0.0042 to 0.0278	0.0079	0.136	0.022
T2LV ($\times 10,000 \text{ mm}^3$)	393	-0.1399	-0.2425 to -0.0372	0.0077	-0.135	0.022
PASAT	393	0.0107	0.0021 to 0.0193	0.0147	0.124	0.019
Whole-brain MTR (mean)	156	0.0319	-0.0101 to 0.0738	0.1352	0.121	0.034
Mean NAA concentration (London) (millimolar units)	133	0.2062	-0.0867 to 0.4992	0.1660	0.121	0.054
MUCCA (mm^2)	128	0.0197	-0.0091 to 0.0486	0.1778	0.120	0.045
NAWM MTR (mean)	156	0.0269	-0.0141 to 0.0679	0.1965	0.104	0.030
MSFC ^a (z-score)	393	0.1358	-0.0011 to 0.2727	0.0518	0.099	0.014
GM MTR (mean)	156	0.0263	-0.0163 to 0.0689	0.2250	0.098	0.029
9HPT (sec^{-1})	393	9.5974	-2.9570 to 22.1518	0.1336	0.076	0.010
BV ($\times 10,000 \text{ mm}^3$)	393	0.0113	-0.0042 to 0.0268	0.1519	0.073	0.009
DGMV ($\times 10,000 \text{ mm}^3$)	393	0.2061	-0.1078 to 0.5200	0.1974	0.066	0.008
EDSS score at randomisation	393	-0.1035	-0.2729 to 0.0659	0.2305	-0.061	0.008
EDSS score at baseline	393	-0.1021	-0.2716 to 0.0675	0.2373	-0.060	0.008
MSWSv2 score	392	-0.0077	-0.0209 to 0.0055	0.2515	-0.058	0.007
MSIS20v2 physical score	393	-0.0067	-0.0183 to 0.0049	0.2550	-0.058	0.007
Mean Glx concentration (London) (mM)	133	-0.0848	-0.3824 to 0.2127	0.5736	-0.049	0.042
MSIS29v2 total score	393	-0.0041	-0.0130 to 0.0048	0.3607	-0.047	0.006
CGMV ($\times 10,000 \text{ mm}^3$)	393	0.0132	-0.0158 to 0.0423	0.3712	0.045	0.006

TABLE 22 Investigation of baseline predictors of PBVC at 96 weeks (*continued*)

Baseline variable	n	Coefficient	95% CI	p-value	Standardised coefficient	R ²
EQ-5D-5L – VAS score	393	-0.0019	-0.0085 to 0.0047	0.5685	-0.029	0.005
EQ-5D-5L – index score	391	-0.1687	-0.9002 to 0.5628	0.6505	-0.023	0.005
T25FW (seconds)	391	-0.0006	-0.0052 to 0.0039	0.7783	-0.014	0.004
MSIS29v2 psychological score	393	-0.0016	-0.0272 to 0.0240	0.9038	-0.006	0.004
LE, left eye; RE, right eye; T2L, T2 lesion. a Statistically significant interaction between baseline variable and amiloride arm ($p < 0.05$). Effect of variable on BV change may be better than implied in this table.						

TABLE 23 Investigation of baseline predictors of EDSS score at 96 weeks

Baseline variable	n	Coefficient	95% CI	p-value	Standardised coefficient	R ²
EDSS score at baseline	383	1.01745	0.92075 to 1.11414	< 0.0001	0.729	0.532
EDSS score at randomisation	383	0.99912	0.90058 to 1.09767	< 0.0001	0.716	0.513
MSWSv2	383	0.06392	0.05489 to 0.07296	< 0.0001	0.582	0.340
MSIS physical score	383	0.0431	0.03424 to 0.05195	< 0.0001	0.443	0.196
MUCCA (mm ²)	131	-0.0411	-0.0596 to -0.0227	< 0.0001	-0.366	0.139
MSIS29v2 total score	383	0.0262	0.01906 to 0.03333	< 0.0001	0.351	0.122
MSFC, ^a z-score	383	-0.3977	-0.3494 to -0.5060	< 0.0001	-0.349	0.122
EQ-5D-5L index score	381	-2.0406	-2.6361 to -1.4451	< 0.0001	-0.330	0.109
T25FW (seconds)	381	0.01145	0.00786 to 0.01503	< 0.0001	0.310	0.096
9HPT (sec ⁻¹)	383	-30.548	-40.607 to -20.489	< 0.0001	-0.294	0.087
NfH (pg/ml)	51	0.00201	1.5E-05 to 0.00401	0.0484	0.285	0.094
SDMT (number of correct answers)	379	-0.0248	-0.0333 to -0.0163	< 0.0001	-0.284	0.081
NfL (pg/ml)	51	0.00046	-0.0002 to 0.00108	0.1416	0.221	0.059
T2LV (× 10,000 mm ³)	383	0.16266	0.06886 to 0.25646	0.0007	0.173	0.031
PASAT (number of correct answers)	383	-0.0112	-0.0186 to -0.0039	0.0030	-0.153	0.024
Mean NAA concentration (London)	136	0.15369	-0.0421 to 0.34943	0.1228	0.135	0.022
DGMV (× 10,000 mm ³)	383	-0.33831	-0.59672 to -0.07990	0.0104	-0.132	0.018
BV (× 10,000 mm ³)	383	-0.01552	-0.02843 to -0.00262	0.0185	-0.121	0.016
CGMV (× 10,000 mm ³)	383	-0.02845	-0.05261 to -0.00430	0.0211	-0.118	0.015
EQ-5D-5L VAS	383	-0.0056	-0.011 to -0.0001	0.0447	-0.104	0.012
Mean myoinositol concentration (London) (millimolar units)	136	0.15008	-0.107 to 0.4072	0.2503	0.101	0.014
SLCVA 2.5% LE (number of correct answers)	377	-0.0074	-0.0157 to 0.00079	0.0762	-0.092	0.009
SLCVA 5% RE (number of correct answers)	373	-0.0071	-0.0151 to 0.00091	0.0823	-0.091	0.009
continued						

TABLE 23 Investigation of baseline predictors of EDSS score at 96 weeks (continued)

Baseline variable	n	Coefficient	95% CI	p-value	Standardised coefficient	R ²
SLCVA 5% LE (number of correct answers)	375	-0.0069	-0.0146 to 0.00089	0.0827	-0.090	0.009
SLCVA 2.5% RE (number of correct answers)	375	-0.0068	-0.0151 to 0.00143	0.1045	-0.084	0.008
Mean Glx concentration (London) (millimolar units)	136	-0.07	-0.2698 to 0.12974	0.4893	-0.061	0.008
HCVA RE (number of correct answers)	376	-0.004	-0.0129 to 0.00479	0.3694	-0.047	0.003
T2L MTR (mean)	156	-0.0084	-0.0368 to 0.02003	0.5610	-0.047	0.032
BV MTR (mean)	156	-0.0061	-0.041 to 0.02877	0.7300	-0.028	0.030
HCVA LE (number of correct answers)	378	-0.002	-0.011 to 0.00696	0.6617	-0.023	0.002
MSIS psychological score	383	0.00472	-0.0174 to 0.02679	0.6744	0.022	0.002
SLCVA 1.25% LE (number of correct answers)	377	-0.0014	-0.0116 to 0.0088	0.7891	-0.014	0.001
GM MTR (mean) (millimolar units)	156	-0.0029	-0.0384 to 0.03259	0.8721	-0.013	0.030
SLCVA 1.25% RE (number of correct answers)	375	-0.0013	-0.0118 to 0.00929	0.8152	-0.012	0.001
NAWM MTR (mean)	156	-0.0019	-0.0362 to 0.03236	0.9130	-0.009	0.029

LE, left eye; RE, right eye; T2L, T2 lesion.
 a Statistically significant interaction between baseline variable and amiloride arm ($p < 0.05$). Effect of variable on BV change may be better than implied in this table.

In *Report Supplementary Material 1, Table 21*, we show the improvement in the model fit when one or both of the baseline CSF neurofilament variables are included in model 1 or 2 (in addition to all the other variables in the models).

The results in *Report Supplementary Material 1, Table 21*, suggest that there is a substantial improvement in both R^2 and adjusted- R^2 after including CSF NfH, even in models with as many as 14 baseline variables. We found that CSF NfH significantly predicted PBVC at 96 weeks (see *Report Supplementary Material 1, Table 22*) in models 1 and 2 even after adjusting for many other variables that were predictive of outcome [standardised coefficients -0.427 ($p = 0.04$) and -0.362 ($p = 0.03$) for models 1 and 2, respectively]; therefore, baseline CSF NfH may have utility in prediction of PBVC at 96 weeks.

Modelling of treatment effect according to baseline magnetic resonance imaging/disability scores

When fitting models including interaction terms, only two baseline variable analyses had a significant interaction term at the 5% level (MSFC and SLCVA 1.25% right eyes) (see *Report Supplementary Material 1, Tables 23 and 24*). No interaction terms were significant at the 1% level.

Surrogate end-point analysis

In the absence of any treatment effect, the results of this modelling are not reported in the monograph.

Chapter 5 Discussion

In this multiarm, double-blind, placebo-controlled Phase IIb trial of three potential neuroprotective agents, we have found that amiloride, fluoxetine and riluzole did not significantly reduce the rate of brain atrophy over 96 weeks in the randomised cohort of 445 patients with SPMS.

From a clinical perspective, none of the measures, including EDSS, MSFC, T25FW, 9HPT, SLCVA, SDMT and PASAT, showed statistically significant or clinically meaningful changes versus placebo. We saw significant differences in some of the PROMs (e.g. MSIS29v2 total and psychological score or NFI nocturnal interval); however, these findings were inconsistent across the trial arms, and we did not feel that they had clinical relevance and were probably due to multiple testing.

The drugs were safe and well adhered to.

A multiarm approach with the simultaneous evaluation of three compounds was effective and efficient and able to be performed in the UK health-care environment with MRI as the primary outcome. The MRI atrophy measure performed as expected with PBVC of -0.65 to -0.70% per year. It effectively allowed three Phase IIb studies to be performed over the trial period – essentially compressing 15 years of work into 5 years. There was excellent buy-in from both the patient community and the neurologists and investigators trying to find a disease-modifying therapy in progressive MS.

Our assessment of the study blinding suggests that blinding was highly successful in this trial.

MS-SMART allowed us to investigate the pathobiological substrate in SPMS. Despite the lack of treatment effect on BV, there may have been focal/multifocal preservation of neural tissue as evidenced by DTI PWSD for RD. In addition, there may have been sparing of neuronal functional health as demonstrated by reduced myoinositol concentration on MR spectroscopy. Notably, these were both seen in the fluoxetine arm. Further analysis of metabolites in tissue-specific volumes is also planned. Moreover, there was a significant decrease in both PBVC at 24 weeks and the number of new/enlarging T2 lesions at 96 weeks from this arm. This is intriguing. However, there was no effect on the primary outcome, and the recently reported results of the FLUOX-PMS trial¹⁰⁸ involving 137 progressive patients treated with fluoxetine/placebo have been negative. Therefore, in the round, and with multiple testing, we do not feel there is a therapeutic effect with fluoxetine.

No treatment effect was seen from the OCT study as a whole.

Limitations to the study would stem from the inherent difficulties in fully understanding the neuropathological processes underlying SPMS. It is rare to obtain brain tissue in life to study and the animal models have well-described drawbacks. One central question is whether or not patients with SPMS should be treated with anti-inflammatory DMTs. When the study was set up and indeed finished, this was not the case in UK standard-of-care practice. Recent Phase III trial data have persuaded the regulatory authorities (Food and Drug Administration and European Medicines Agency) that siponimod should be used in SPMS. We would anticipate that this would be the standard of care (after NICE consideration) in the next 1–2 years and future trials would add on potential neuroprotective agents. That being said, the underlying ‘inflammatory’ activity in our cohort was low, with the median number of new T2 lesions being zero and about 10% of participants having an ‘on-study’ relapse. Of course, the study was not set up to measure other inflammatory parameters such as microglial activity. In retrospect, to determine the proportion with gadolinium enhancement at baseline would have been instructive. Finally, the question of how to trial patients who are not suitable for DMT (e.g. those who fail the NICE criteria) will remain.

There were some missing data on the primary outcome, but this seems to be robust to the sensitivity analyses performed.

In addition, the CSF cohort was small ($n = 39$) and this limits interpretation. Going forward it would seem unlikely that the UK population will participate in large numbers in CSF studies in SPMS and we would favour the development of blood markers. This looks to be achievable with new SIMOA® (Quanterix Corporation, Billerica, MA, USA) based technology.

Chapter 6 Conclusions

The MS-SMART was ground-breaking in its design and its approach to an intractable neurodegenerative disease. It demonstrated that this type of highly efficient trial can be performed in progressive MS, and supports its application in further trials in progressive MS and potentially other neurodegenerative environments. This will enable the research community to accelerate the testing of drugs in these very demanding situations, which have large health-care costs and burdens associated with them.

The trial was well powered, blinding was robust, adherence was high and retention was high. In addition, the primary outcome progressed as expected in the placebo arm, in a similar way to what has been observed in other recent trials in SPMS.^{13,101,109} Valuable information was obtained across the board for all secondary and exploratory measures, which will help to decide their place in future trial design as indicative and mechanistic measures.

Drug selection was performed through a rigorous and systematic process. Proof of concept that this process conveyed translational predictive value is evidenced by the fact that two of the shortlisted compounds have subsequently been demonstrated to exhibit neuroprotective effects in progressive MS at phase 2.^{109,110}

In summary, MS-SMART has laid down the template for future phase 2 drug testing in neurodegenerative disease.

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All authors have approved the final version of the report to be published.

Publications

Journal articles

Chataway J, De Angelis F, Connick P, Parker RA, Plantone D, Doshi A, John N, *et al.* Efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis (MS-SMART): a phase 2b, multiarm, double-blind, randomised placebo-controlled trial [published online ahead of print January 22 2020]. *Lancet Neurol* 2020.

Connick P, De Angelis F, Parker RA, Plantone D, Doshi A, John N, *et al.* Multiple Sclerosis-Secondary Progressive Multi-Arm Randomisation Trial (MS-SMART): a multiarm phase 1b randomised, double-blind, placebo-controlled clinical trial comparing the efficacy of three neuroprotective drugs in secondary progressive multiple sclerosis. *BMJ Open* 2018;8:e021944.

Marshall I, Thrippleton MJ, Bastin ME, Mollison D, Dickie DA, Chappell FM, *et al.* Characterisation of tissue-type metabolic content in secondary progressive multiple sclerosis: a magnetic resonance spectroscopic imaging study. *Neurol* 2018;**265**:1795–1802.

Conference abstracts

Chataway J, Chandran S, Miller D, Connick P, Giovannoni G, Pavitt S, *et al.* (2016). *Recruitment in Multiple Sclerosis Trials: The MS-SMART Experience*. Presented at the Annual Meeting of the Association of British Neurologists (ABN), Brighton, UK, 17–19 May, 2016.

Connick P, Miller D, Pavitt S, Giovannoni G, Wheeler-Kingshott C, Weir C, *et al.* *MS-SMART Trial Design and Recruitment Status: A Multi-arm Phase IIB Randomised Double Blind Placebo-controlled Clinical Trial Comparing the Efficacy of Three Repositioned Neuroprotective Drugs in Secondary Progressive Multiple Sclerosis*. Presented at the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), London, 14–17 September 2016.

De Angelis F, Cameron J, Connick P, Miller DH, Pavitt S, Giovannoni G, *et al.* *Optical Coherence Tomography in Secondary Progressive Multiple Sclerosis: A Baseline Data Report from the MS-SMART Trial*. Presented at the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS), London, 14–17 September 2016.

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Calvi A, Prados F, Tur C, De Angelis F, John N, Doshi A, *et al.* *Characterizing the Slowly Evolving Lesions (SELs) in a Cohort of Secondary Progressive Multiple Sclerosis Patients*. Presented at the 4th Congress of the European Academy of Neurology (EAN), Lisbon, 16–19 June 2018.

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Ammoscato F, De Angelis F, Plantone D, Parker RA, Wheeler-Kingshott CAMG, Pavitt S, *et al.* *CSF Neurofilament Heavy Chain Release (NfH) is Important in Secondary Progressive MS (SPMS) – Results from the MS-SMART Trial*. Presented at the 35th Congress of the European Committee for Treatment and Research in Multiple Sclerosis (ECTRIMS)/24th Annual Conference of Rehabilitation in MS (RIMS), Stockholm, 11–13 September 2019.

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Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to available anonymised data may be granted following review.

Patient data

This work uses data provided by patients and collected by the NHS as part of their care and support. Using patient data is vital to improve health and care for everyone. There is huge potential to make better use of information from people's patient records, to understand more about disease, develop new treatments, monitor safety, and plan NHS services. Patient data should be kept safe and secure, to protect everyone's privacy, and it's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data are used. #datasaveslives You can find out more about the background to this citation here: <https://understandingpatientdata.org.uk/data-citation>.

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